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METASTASIS OF MIXED TUMORS OF THE SALIVARY GLANDS

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From a survey of the literature on mixed tumors of the salivary glands which were proved at autopsy to have metastases it would seem at first glance that the development of secondary growths from them is uncommon as contrasted with the rather high recurrence rate. Although this paper is concerned only with cases authenticated at reasonably complete autopsies, several factors suggest that metastasis of mixed tumors of the salivary glands is more frequent than currently assumed. These factors are: (1) reports of cases with strong presumptive clinical evidence of metastases; (2) reports of cases in which biopsy of lesions distant from the primary site during life showed growths that resembled the primary tumor in every histologic detail; (3) occasional partial autopsy reports of such instances; (4) the difficulty of following the patient to the time of death because of the long clinical course of the disease; (5) the possibility of death of the patient before dissemination could occur; (6) the ever present obstacle of obtaining permission for an autopsy from the relatives of a patient who has suffered from the operative and irradiation aftermaths of treatment for the primary tumor and its recurrences, and (7) the possible failure to record cases with metastases observed at complete autopsies.

In the present analysis of recorded instances of metastasizing mixed tumor of the salivary glands, the following details will be considered: sex, age, location of the primary tumor, duration of the primary tumor before it was first seen by the author, its largest size, duration of life after it was first seen, number of recurrences, location of metastases, histologic characteristics of the primary tumor and of the metastases and the immediate cause of death. When any of these details is not given, it was not found in the original reports.

Although the original reports reveal a rather wide variety of names and thus an apparent dissimilarity of the tumors which are now being considered under the heading of metastasis of mixed tumors of salivary glands, it is thought from the information available that the cases studied are bona fide instances of such metastasis. The histologic variation in

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a group of mixed tumors of the salivary glands, the different descriptive terms which may be employed if their fundamental nature is forgotten and the confusion in the minds of persons who reported cases in the older literature, especially when the eminent authorities of the day disagreed so diametrically on the origin and nomenclature of these tumors, have been borne in mind in the analysis.

REVIEW OF CASES RECORDED IN THE LITERATURE

Förster [1] reported the case of a woman 34 years old who had had a tumor of the right parotid region for eight years. It had attained the size of a goose egg. There was no recurrence, as the patient died from pneumonia a few days after her first visit. Metastases were found in the petrous portion of the right temporal bone, in the lungs and in the pleurae. Sarcomatous, cartilaginous and mucoid tissue comprised the tumor.

Tommasi [2] described a woman 51 years old who had had a tumor in the right submaxillary region for eight years; it had reached the size of a hen's egg. No recurrence was noted. Metastases were discovered in the cervical lymph nodes, pleurae, lungs, liver (12 pounds [5.4 Kg.]), left kidney, pelvic peritoneum, ovaries and dura. They were composed of mixed tumor with a cylindromatous structure.

Chiari [3] observed a man of 45 years with a tumor of the left parotid region of two years' duration, which became the size of a man's fist. The man died of erysipelas and pneumonia three weeks later, without recurrence. The only metastases were in the lungs. The tumor consisted of glandlike tissue and fibrous connective tissue, similar to the so-called cylindroma of the parotid region and orbit.

Griffini and Trombetta [4] saw a woman of 56 years with a tumor of the submaxillary gland which had been present for sixteen years. Metastases were found in the cervical and bronchial lymph nodes, the lungs and the pleurae. The tissues revealed a cellular mixed tumor with a rich content of cartilage and an alveolar arrangement of tumor cells.

Zahn [5] discussed a man 49 years old who had had a tumor of the left submaxillary region for two months. He died of lobar pneumonia four months later, without recurrence. Metastases involved the manubrium of the sternum, the right seventh rib, the second lumbar vertebra and the right hip joint, femur and pubis. The tumor extended into the right anterior cranial fossa, the mouth, the organs of the neck and the right orbit. The tumor cells were in an alveolar pattern and were associated with heavy fibrous tissue. The cells centrally placed were degenerated, and those peripherally located were well preserved.

LeDentu [6] examined a woman 61 years old in whom there had been a tumor of the left parotid region for thirty years, which had attained the bulk of 1,300 Gm., or more than half the size of the patient's head. At autopsy, twenty-one days later,

1. Förster: Wien. med. Wchnschr. **8**:481, 1858.
2. Tommasi, C.: Virchows Arch. f. path. Anat. **31**:111, 1864.
3. Chiari, H.: Wien. med. Presse **21**:746, 1880; Med. Jahrb. **11**:1, 1881.
4. Griffini, L., and Trombetta, F.: Arch. per le sc. med. **7**:71, 1883-1884; cited by Kornblith.[13]
5. Zahn, F. W.: Virchows Arch. f. path. Anat. **117**:1, 1889.
6. LeDentu, A.: Études de clinique chirurgicale, Paris, Masson & Cie, 1892, p. 154; cited by Heinike, H.: Ergebn. d. Chir. u. Orthop. **6**:239, 1913.

metastases were found in the lungs, liver and the meninges. The tissue consisted of fibrous septums about irregular alveoli. Some areas were in the process of liquefaction.

Barozzi and Lesne [7] saw a woman 34 years old who had had a tumor of the left submaxillary region for twelve years. Metatases were noted in the cervical and the tracheal lymph nodes and in the lungs. The tissue showed connective tissue surrounding islands of myxoepithelioma and the pattern of cylindroma.

LeClerc [8] described a man 65 years old who had had a tumor of the right parotid gland for four years, which became the size of an orange. Death was caused by extension of the tumor to the right zygoma, the right middle cerebral fossa, the right temporal lobe and the right orbit. Metastases were found in the lungs and pleurae. He called the tumor a malignant glandular epithelioma.

Rispal and Samiac [9] observed a 62 year old woman with a tumor in the left parotid region. She died from the effects of its extension to the dura six weeks later. Metastases were present in the cranial bones. The microscopic diagnosis was cylindroma.

Partsch [10] saw a woman 31 years old who had had a tumor in the right parotid region for sixteen years; it had reached the size of a hen's egg. She lived for thirteen years, and two recurrences developed. Metastases were noted only in the pleurae after death from cellulitis of the right cheek and neck, which developed after an operation for the second recurrence. The specimen was a well encapsulated mixed tumor of cylindromatous structure with extensive hyaline and mucous transformation.

Garvey [11] reported the case of a woman 31 years old who had had a tumor in the left parotid region for eight years, which attained the size of an olive. She died eighteen months later of dilatation of the right side of the heart with passive congestion of the viscera, after experiencing two recurrences in the course of the disease. Metastases were found in the vertebrae, femurs, skull, sternum, clavicle, lungs, pleura, liver, retroperitoneal lymph nodes, adrenals and spinal meninges. The tumor also extended into the left gasserian ganglion. It was diagnosed as adenocarcinoma.

Brunschwig [12] discussed a woman 60 years old who had had a tumor of the right sublingual gland for nineteen years. She had one recurrence. A few days after her last admission to the hospital she died from the effects of the tumor. The lungs and pleura were involved by metastases. The tumor extended to the mandible, the mouth and the larynx. The four photomicrographs indicate its mixed tumor nature. The author commented: "In all these sections made from tissue removed at intervals over a period of 19 years, the character of the epithelial elements remains unchanged."

Kornblith's first case [13] was that of a man 55 years old with a tumor in the right submaxillary region the size of a plum. Three recurrences marked the total

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7. Barozzi and Lesne: Bull. Soc. anat. de Paris **72**:266, 1897; cited by Kornblith.[13]

8. LeClerc, G.: Lyon méd. **101**:864 and 900, 1903.

9. Rispal and Samiac: Toulouse méd. **8**:121, 1906.

10. Partsch, F.: Deutsche Ztschr. f. Chir. **183**:269, 1923.

11. Garvey, J. L., in Stone, W. J.: Contributions to Medical Science, Dedicated to Aldred Scott Warthin, Ann Arbor, Mich., George Wahr, 1927, p. 661.

12. Brunschwig, A.: Surg., Gynec. & Obst. **50**:407, 1930.

13. Kornblith, B. A.: Virchows Arch. f. path. Anat. **286**:74, 1932.

course. Slightly over four years after his first visit he died. The lungs and the liver contained metastases. The structure of a cylindroma was evident. Small, delicate epithelial cells were arranged in nests in a connective tissue or mucous tissue stroma. Hyaline masses were in the centers of the cell groups.

Fitzwilliams[14] observed a woman 48 years old in whom a tumor of the left submaxillary region had been present for five years. She had two recurrences. Autopsy, four months after the author first saw her, disclosed metastases in the cervical lymph nodes on the left side and in the pleurae and extension to the mandible. The structure was that of a cellular salivary gland tumor. Polygonal cells formed small solid masses; columnar cells formed tubes and alveoli; cells were drawn out in a mucinous stroma; a small number of mitotic figures was seen.

Boemke[15] examined a woman 35 years old in whom a tumor of the hard palate had been present for five years. Two days after operation for removal of the first recurrence, she died of bronchopneumonia. The tumor and the metastases in the lungs, the liver and the right kidney was a cylindroma with the formation of hyaline cylindromatous structures.

Olson[16] outlined the case of a woman 49 years old who had a tumor of the right side of the hard palate and metastases in the bronchial and mediastinal lymph nodes, the lung, the pleurae, the spleen and the liver, which he designated a mixed tumor.

Kornblith's second case[17] was that of a woman 33 years old who had had a tumor in the right parotid region for fourteen years. It had attained the size of a plum. She had one recurrence and died a few months after the author first saw her of the effects of the tumor, which extended to the mandible, the zygoma, the temporal bone, the middle and posterior cranial fossae and the rhinopharynx; compressed the cerebrum, the pons and the medulla; and metastasized to the cervical lymph nodes, the lungs, the pleura of the left diaphragm and the liver. The tumor was composed of "branching, interlacing, homogeneous, hyaline, mucoid, cylindrical rows of globules lined by small cells in single or double rows and resembling basal cells." It had the structure of a cylindroma.

McKnight[18] observed a Negro boy 15 months old with a tumor of the left parotid region present since birth; it had reached the size of a plum. One recurrence followed operative removal, ten months after which the boy died from the effects of the tumor. Metastases were discovered in the skull bones, the left temporal lobe of the brain and the lungs. The tumor was diagnosed as adenocarcinoma.

Livingston[19] reported the case of a Negro man 44 years old who had had a tumor in the right parotid gland removed nine years prior to his last admission. Six years after that operation a recurrence was seen. Ten weeks after his last admission he died of lobar pneumonia. The lungs, liver, spleen, left adrenal, gallbladder, sternum and left second rib were involved by metastases. The tumor was diagnosed as "teratoma (mixed tumor)." It was composed of fairly uniform, lightly staining polyhedral cells. Mitotic figures were present but not numerous. In places the cellular structure presented a mucoid appearance.

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14. Fitzwilliams, D. C. L.: Lancet **2**:769, 1935.
15. Boemke, F.: Centralbl. f. allg. Path. u. path. Anat. **64**:129, 1936.
16. Olson, G. W.: Laryngoscope **47**:252, 1937.
17. Kornblith, B. A.: J. Mt. Sinai Hosp. **6**:38, 1939.
18. McKnight, H. A.: Am. J. Surg. **45**:128, 1939.
19. Livingston, S. K.: Am. J. Roentgenol. **44**:887, 1940.

Perrin [20] described a man 35 years old who had had a tumor of the right parotid region for seven years and who died four months later. Metastases were found in the lungs, liver, pleurae, lumbar vertebrae, subcutaneous tissue, omentum and retroperitoneal tissue. A matrix of hyalin and mucocartilage contained cell strands and aggregates of glands of juvenile cubical epithelium. In some areas were hollow branching formations of neoplastic epithelium with hyperchromatic nuclei.

A number of case reports of histologically proved distant metastases, such as those by Budde [21] and by Gueit and Puech,[22] have appeared in the literature. Several investigators have described cases with histologically substantiated metastases in lymph nodes. Others have observed cases in which all other primary sites were eliminated on clinical grounds. In the tumor clinic here 2 patients have been followed who had metastases in the lungs as shown by roentgen examination, with probable origin in the salivary glands, but unfortunately permission for autopsy was not obtained in either case. None of these cases is included in the present report, since the studies made of them are incomplete in that other possible primary sites of origin were not eliminated.

REPORT OF A CASE

A bookkeeper 54 years old was admitted to the Colorado General Hospital on March 22, 1942. For twelve years he had had a tumor the size of a pea anterior to the left ear in the region of the parotid gland. In the course of a year this enlarged to the size of a lemon. Six months before admission, while on a fishing trip he fell down in a boat. Following this, he experienced lumbar pain which radiated down the right leg. He received a "spinal adjustment" and massage of the parotid tumor. Temporary lessening of the pain and some decrease in the size of the tumor were noted. For four months he had paralysis, first of the right and then of both legs, necessitating confinement to bed. He also had uncontrollable dribbling of urine. For three months he tolerated only a soft diet because of gagging caused by ingestion of solid food. For four days he was drowsy.

On examination the temperature was 98 F.; the pulse rate, 110; the respiratory rate, 20. The blood pressure was 116 systolic and 80 diastolic. In addition to drowsiness and gagging at the sight of a tongue blade (making pharyngeal examination impossible), the significant findings were: anterior to the left ear, a nontender hard lemon-sized mass attached to the underlying tissue but not to the skin; false dentures; bilateral foot drop with paralysis of the flexor muscles and marked paresis of the extensor muscles; marked incoordination in the heel to knee test; marked weakness in the thigh and hip muscles; absence of patellar and achilles reflexes; bilateral absence of the Babinski sign; normal sensation and vibratory sense. On neurologic consultation the diagnosis was: compression of the cauda equina by a metastatic tumor.

The hemoglobin was 15.0 Gm. (Newcomer); the erythrocyte count, 5,280,000; the total leukocyte count, 12,160, with polymorphonuclear neutrophils 68 per cent, lymphocytes 30 per cent and monocytes 2 per cent. The sedimentation rate was 22 per cent in one hour. The urinalysis, the Wassermann test of the blood and

20. Perrin, T. L.: Arch. Path. **33**:930, 1942.

21. Budde, M.: Zentralbl. f. Chir. **49**:1888, 1922.

22. Gueit and Puech: Bull. Soc. d. sc. méd. et biol. de Montpellier **4**:52, 1922-1923.

the Eagle test gave negative results. The spinal fluid contained no cells; the sugar content was 72 mg. and the protein content 88 mg. per hundred cubic centimeters; the Wassermann reaction was negative, as were the gold curve and the culture. The sugar content of the blood was 99 mg. and the nonprotein nitrogen content 39 mg. per hundred cubic centimeters.

Roentgen examination of the skull revealed no definite change; that of the chest, accentuated hilar and bronchial markings, round areas of increased density in the middle thirds of the lung fields and accentuation of the aortic arch; that of the pelvis and of the lumbar part of the spinal column, extensive destruction with wedging and anterior displacement of the fourth lumbar vertebra, erosion of the body of the third lumbar vertebra and ballooning of the intervertebral disks in the lumbar part of the spinal column, general atrophy of all bones visualized, mottling of the femur and of the pelvic bones; that of the gastrointestinal tract, no definite evidence of an organic lesion in the stomach or the duodenum. There was a marked six hour gastroduodenal residue.

He continued to be drowsy, became irrational and had hallucinations and delusions. The temperature ranged between 98 and 100 F.; the pulse rate, betwen 90 and 120. He was still unable to tolerate any but liquid food and was nauseated. He was seen by the members of the staff of the Bonfils Foundation Tumor Conference March 25. The impression was one of widespread metastases with origin possibly in the left parotid region. A punch biopsy, suggested then, was made April 2. Histologic examination revealed groups of polyhedral cells with scanty acidophilic cytoplasm and dark oval or round nuclei. Many mitotic figures were seen. The cells were occasionally grouped to form tubules containing pale basophilic material. April 7 the temperature rose to 103 F., the pulse rate to 170 and the respiratory rate to 34. Moist rales were heard throughout the chest. The patient died April 8, seventeen days after admission and six days after biopsy.

Postmortem Examination.—Autopsy, sixteen and one-half hours after death, disclosed a bulging tumor, 6 by 4.5 cm., in the left parotid region. On section this was hard, glistening, yellow-white and studded with pinpoint gelatinous areas. At its lower pole was a red-yellow firm 8 mm. nodule.

Infiltrating beneath the pleura of the anterior ends of the fifth and sixth ribs on the right and the third and seventh ribs on the left was a pink-gray nodular tumor. The largest area was 2.5 cm. The overlying pleura was smooth and intact, although the shafts of the corresponding ribs were invaded.

The heart weighed 380 Gm. The foramen ovale was anatomically patent. The septum primum covered the posterior half and overlapped the septum secundum, but a slit 1 cm. long was demonstrated by probing. The septum primum was marked by six fenestrations, each 0.5 mm. in diamter, on its anterior superior edge. The valves all had thin, delicate leaflets. The thickness of the right ventricle was 3 to 4 mm.; that of the left ventricle, 15 to 18 mm. The coronary arteries in the first two thirds of their extent were stiffened by yellow and white intimal plaques, but no occlusion was seen.

The right lung weighed 810 Gm.; the left, 610 Gm. The surfaces were dark red and smooth and finely mottled with black. Multiple tumor nodules bulged beneath the pleura. A cut section of the posterior four fifths of the lungs was dark red, finely mottled with black and noncrepitant; the anterior one fifth was cushiony, pink-gray, subcrepitant. The anterior edges and apexes showed emphysematous bullae, 4 to 5 cm. in diameter. The tumor nodules, most of them subpleural, 7 to 25 mm. in diameter, were discrete, rounded, firm, red-yellow and

studded with pinpoint glistening gray dots. The trachea and bronchi contained thick gray and yellow and frothy pink-gray fluid.

The stomach was distended with air and about 1,000 cc. of glairy gray-yellow opaque fluid. The mucosa was moderately flattened, pale and gray-yellow. Beneath the mucosa of the colon were gray, red-rimmed nodules up to 5 mm. in diameter. In the sigmoid colon was a pedunculated 1 cm. polyp with a thin stalk.

The liver weighed 1,520 Gm. The cut surface was firm and brown-red. It contained seven pink-white to red-yellow nodules, 5 to 20 mm. in diameter.

As to the skeleton, the anterior end of the bony portion of the left seventh rib was fractured through expansion of its medulla by tumor invasion. The first, second, third and fourth lumbar vertebrae were removed in right hemisection. The body of the second was softened and mushy. The fourth was almost completely destroyed by friable glistening red-yellow tumor, which extended symmetrically on either side beneath the iliopsoas muscles and encroached on the cauda equina.

The brain weighed 1,800 Gm. The convolutions were flattened. Routine transverse sections through it revealed no gross abnormalities.

Microscopic Examination.—The significant findings aside from those in the tumor and its metastases were: early bronchopneumonia, chronic passive congestion of the lungs and the liver, early acute esophagitis, melanosis and noncancerous polyp of the colon, moderate adipose tissue replacement of the pancreas and acute lymphadenitis of the mediastinal nodes.

Tumor of the Left Parotid Gland (*A* and *B* in figure): The tumor cells were polyhedral. Their cytoplasm was acidophilic. The nuclei were round or oval and had a heavy stippled chromatin content. Mitotic figures were evident. Large and small cylinders of amorphous basophilic material were scattered among the tumor cells, which were arranged in plaques of varying size set in a pale basophilic stroma enclosing a few flat spindle cells. The basophilic stroma was separated into islands by broad strands of collagenous connective tissue, in which a rare focus of pigmented macrophages was seen. The tumor impinged on the hilus of a well preserved lymph node, adjacent to which was tissue of the normal left parotid gland (*A* in figure). In the substance of the lymph node were several small epithelial tubules (*C* in figure) which were similar in their structure to the ducts of the nearby parotid gland. The inclusion of tubules in this fashion was described by Neisse.[23]

Metastases in the Lung, Liver and Kidney (*D* and *E* in figure): These consisted of polyhedral cells with acidophilic cytoplasm and round or oval nuclei with dense stippled chromatin content, among which were scattered mitotic figures. The tumor cells were supported on a delicate, well vascularized stroma. Many large and small cylinders of basophilic material were surrounded by tumor cells. The nearby lung and liver parenchyma was compressed and atrophied.

In the kidney was a focus of tumor with a structure like that of the growths in the lungs and the liver. A mass of tumor cells plugged a neighboring vein.

Fourth Lumbar Vertebra (*F* in figure) and Adjacent Soft Tissue: The tumor cells were identical with and arranged in patterns like those in the other metastases except that wide areas of mucoid degeneration were evident within the cylinders among them and in the zone surrounding islands of tumor cells. Adjacent to this outer zone of mucoid change, heavy bands of interlacing connective and collagenous tissue contained blood vessels, nerve trunks, many pigmented macrophages and two small islands of bone.

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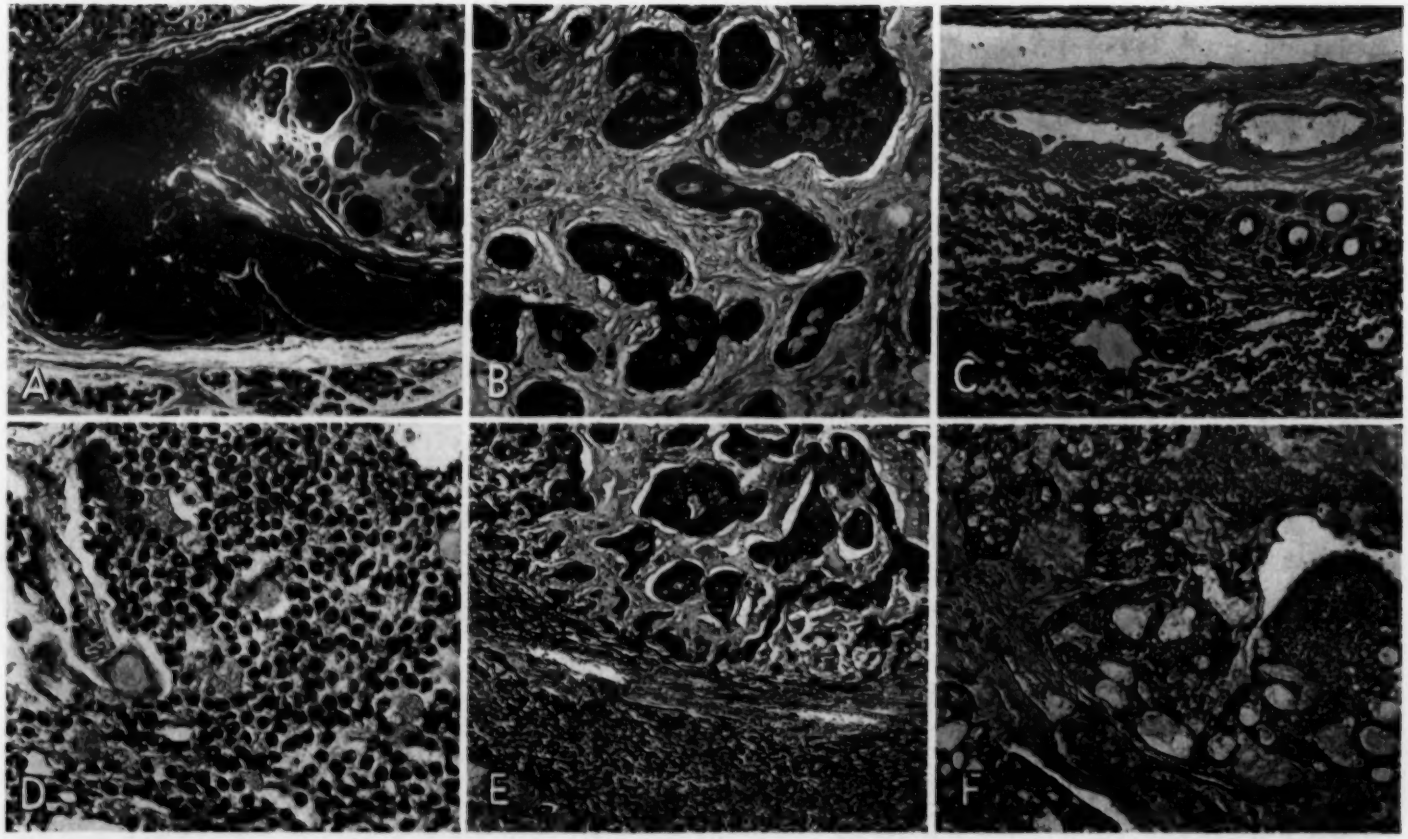
23. Neisse, R.: Anat. Hefte **10**:287, 1898.



A, impingement of the primary tumor on the hilus of a lymph node. Note the normal parotid gland just beneath the lower edge of the node. × 12. *B*, detail of primary tumor. × 125. *C*, epithelial tubules like those in the parotid gland caught in the hilar edge of the lymph node seen in *A*. × 125. *D*, detail of a tumor metastasis in the lung. × 250. *E*, tumor nodule with compression of the liver parenchyma in the lower half of the field. Note the periportal area in the lower left hand corner. × 50. *F*, metastasis in the fourth lumbar vertebra. × 50.

The final anatomic diagnosis was: cancerous mixed tumor of the left parotid gland with metastases to the pleurae, lungs, liver, kidneys, ribs and lumbar vertebrae; coronary arteriosclerosis; cardiac dilatation; patent foramen ovale; cerebral edema; chronic passive congestion of the lungs and liver; bronchopneumonia; acute lymphadenitis of the mediastinal lymph nodes; acute esophagitis; noncancerous polyp and melanosis of the colon.

ANALYSIS OF DATA COLLECTED IN TWENTY-ONE CASES

In 20 cases the age at which the patient was first seen by the author varied from 31 to 65 years. Eight males and 13 females were afflicted. In 12 cases the tumor was in the parotid region; in 6, in the submaxiliary region; in 2, in the hard palate, and in 1, in the sublingual gland. The duration of the tumor before it was first seen by the author in 19 of the cases was two months to nineteen years. In 11 cases the largest size ranged from that of an olive to more than half the size of the head. The duration of life after the patient was first seen by the author in 17 cases was from two days to thirteen years. The recurrences ranged from none to three. Of the entire series, metastases were present in the lungs in 18, in the pleurae in 12, in the liver in 10, in the bones in 8, in lymph nodes in 6, in the kidneys in 3 and in the spleen in 2. In 8 cases the term "cylindroma" was employed in the histologic description of the tumor. In 4 cases this same appearance was present if one may judge from the description given. In 5 "mixed tumor" was the designation of the neoplasm. In 2 "adenocarcinoma" was the term used. In the 2 remaining cases "malignant glandular epithelioma" and "alveolar sarcoma" were the names given to the growths.

COMMENT

The facts presented in the foregoing analysis of the data in 21 cases suggest that the following points should be remembered when a patient in the fourth, fifth or sixth decades of life has a tumor of a salivary gland: duration of the growth; size of the tumor at various stages in its evolution; a consideration of the mestastases as they may affect the lungs, pleurae, liver, bones, lymph nodes, kidneys, spleen and other sites; a careful determination of the extension of the tumor, particularly into the meninges and the brain; some thought of the possible relationship of a cylindromatous type of structure in the primary tumor to the development of metastases, and a thorough examination of the patient to rule out metastases if an operation on or irradiation of the primary tumor is contemplated.

SUMMARY

A review has been made of 20 case reports of mixed tumors of the salivary glands in which metastases were found at autopsy.

lumbar vertebra. × 50.

COMMERCIAL LEAD AS A POSSIBLE INCITING FACTOR IN BRONCHIOGENIC CARCINOMA

REPORT OF TWO CASES

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It is evident from the literature that little or no emphasis is placed on commercial lead as a factor in the production of primary carcinoma of the lung. Probably not enough care is directed to the incidence of carcinoma in occupations that deal with commercial lead. Lanza [1] emphasized the inadequacy of information on the mortality from lead poisoning and the absence of information relative to the morbidity. He stated that there is no doubt that in many cases lead poisoning is reported on the death certificate as some organic disease, no mention being made of the underlying plumbism. Lanza [1] also explained why the manifestations of plumbism are less frequently encountered in the major lead industries, such as smelters and battery and paint factories, but are seen in industries where the contact with commercial lead is just one step in the manufacture. This is because the heads of the major industries are fully cognizant of the hazards of lead taken into the body and utilize precautionary measures against it, while those of industries and occupations in which the contact with lead is only a step in the process tend to underestimate the hazard or to ignore it completely. It is likewise difficult to obtain accurate information in regard to such varied occupations as those of painters, printers and plumbers.

The widespread and increasing use of lead throughout industry makes it of more and more concern to the physician and the public health official. Some of the industries in which lead is a hazard are: lead mining; lead smelting; lead refining; lead fabrication; hot lead processes; lead burning; lead soldering; lead tempering; plumbing; iron and other founding; buffing and polishing; storage battery, paint, glass, rubber and chemical industries; the applying and removing of lead paints, enamels and glazes, and typographic trades, such as type founding, electrotyping and stereotyping. According to Lanza,[1] most of the industrial exposure arises from the inhaling of dust and fumes into the lungs.

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1. Lanza, A. J.: J. A. M. A. **104**:85, 1935.

Several writers have reported pulmonary carcinoma in workers of the glass industry, in which lead is used. Lead is employed mainly in the manufacture of flint glass, which is used in table and other fine glassware and in high grade optical lenses. Lead oxide is commonly used in flint glass in the proportion of 33 per cent. Boyd,[2] as early as 1887, reported cancer of the lung in a glass blower. Gutzeit [3] reported bronchiogenic carcinoma in a glass maker. Klotz [4] reported carcinoma of the lung in 24 persons, among whom were 2 glass workers, 1 miner and 1 polisher. Metal polishes often contain lead. Seyfarth [5] noted that carcinoma of the lung occurred with special frequency among cigar makers, metal workers, type setters and printers. According to Kohn,[6] some of the intoxications associated with tobacco have been found to be due to lead.

Rosedale and McKay [7] reported a study of the occupational incidence of carcinoma of the lung. Forty-three patients, or 75.4 per cent of the 57 persons studied, were employed in occupations exposing them to dust or other irritating atmospheric factors, and 9 of the 43 had been working more or less with lead in one form or another. Lanza [1] pointed out that lead poisoning is not uncommon among industrial workers who are not engaged in handling lead but who through negligence are exposed to dust or fumes from lead processes not properly protected.

There is much difference of opinion as to the role played by the various dusts and fumes in inciting carcinoma of the lung. The view that tuberculosis and influenza are factors in the production of bronchiogenic carcinoma is gradually being discarded. Weller [8] summarized the causes by stating that a variety of chronic factors are potent in the production of carcinoma of the lung in various degrees in different persons, depending on the degree of intrinsic predisposition.

It is generally agreed that inhaling radioactive dust is an important etiologic factor in carcinoma of the lung. Saupe,[9] in a critical review, concluded that the high incidence of pulmonary carcinoma in the Schneeberg cobalt miners in Europe over a long period of years was due to inhalation of radioactive mine dusts. Saupe [9] also emphasized that carcinoma develops after prolonged exposure to radioactive dust, ranging from ten to forty-five years, the average age of incidence being 55 years.

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2. Boyd, M. A.: Lancet **2**:60, 1887.
3. Gutzeit, K.: Ztschr. f. Krebsforsch. **19**:30, 1922.
4. Klotz, O.: Canad. M. A. J. **17**:989, 1927.
5. Seyfarth, C.: Deutsche med. Wchnschr. **50**:1497, 1924.
6. Kohn, H.: Deutsche med. Wchnschr. **52**:447, 1926.
7. Rosedale, R. S., and McKay, D. R.: Am. J. Cancer **26**:493, 1936.
8. Weller, C. V.: Arch. Path. **7**:478, 1929.
9. Saupe, E.: Zentralbl. f. inn. Med. **54**:825, 1933.

Weller,[8] Fried,[10] Vinson,[11] Rice,[12] Stein and Joslin,[13] Matz,[14] Rosahn,[15] Ochsner and DeBakey,[16] Menne and Anderson [17] and Karsner [18] could find no definite cause for bronchiogenic carcinoma except for that in the Schneeberg cobalt miners, which is thought to be due to the radioactive dust in the atmosphere of the mines.

In chemical character lead and radium are closely allied, since they belong to the same chemical family. Their atomic numbers are in the same range in the periodic table, radium being 88, radon, 86, radium D 82 and lead 82. This further denotes similar chemical properties. In fact, radium is only one member of the family of linear descendants from the parent element, uranium, to the various radioactive isotopes of lead, such as radium B, thorium B, actinium B and radium D, or radiolead (Hoffman and Strauss). According to Rutherford and co-workers,[19] radium D is so closely allied physically and chemically to lead that it cannot be isolated in the analysis of a uranium mineral. They emphasized that if any radium is present in the ore from which lead is prepared, radium D will be separated with the lead and remain with it throughout the process of purification. They stated that the life span of radium D is about twenty-five years, meaning that the radioactivity of commercial lead decreases with age. Smith and associates [20] recently reemphasized that commercial lead is by far the most radioactive of all the common metals. Certain lots of industrial lead contain more radioactive substances than others, depending somewhat on where the ore is mined.

The toxic manifestations of lead and radium are strikingly similar; radium, being more toxic, produces a more marked effect. Smith and co-workers [20] and others have shown that colloidal lead preparations containing radioactive lead when injected into laboratory animals caused ulceration of the jaw, degenerative changes in the liver and the kidneys and inflammatory infiltrations of the lungs. The lesions of the bones, such as rarefaction due to necrosis and osteitis, were similar to those

10. Fried, B. M.: Medicine **10**:373, 1931.
11. Vinson, P. P.: J. A. M. A. **107**:258, 1936.
12. Rice, C. M.: J. Lab. & Clin. Med. **21**:906, 1936.
13. Stein, J. J., and Joslin, H. L.: Surg., Gynce. & Obst. **66**:902, 1938.
14. Matz, P. B.: J. A. M. A. **111**:2086, 1938.
15. Rosahn, P. D.: Arch. Path. **29**:649, 1940.
16. Ochsner, A., and DeBakey, M.: Arch. Surg. **42**:209, 1941.
17. Menne, F. R., and Anderson, M. W.: J. A. M. A. **117**:2215, 1941.
18. Karsner, H. T.: Human Pathology, ed. 6, Philadelphia, J. B. Lippincott Company, 1942, p. 479.
19. Rutherford, E.; Chadwick, J., and Ellis, C. D.: Radiations from Radioactive Substances, New York, The Macmillan Company, 1930, pp. 519 and 539.
20. Smith, F. L.; Rathmell, T. K., and Marcil, G. E., in Piersol, G. M., and Bortz, E. L.: The Cyclopedia of Medicine, Surgery and Specialties, ed. 2. Philadelphia, F. A. Davis Company, 1940, vol. 8, p. 811.

produced by radium. Crawford and associates,[21] experimenting with cats, found that those which received colloidal lead alone generally remained in good clinical condition without loss of weight and without anemia. However, the cats which received both colloidal lead and radiation had more pronounced manifestations, such as loss of weight, anemia and infections, than the group of cats that received lead alone or radiation alone. The tissues examined from the cats which received only lead showed no characteristic changes. These observations are in accord with those of Aub [22] and the opinion of most pathologists that there is no specific lesion produced by lead with any degree of frequency.

Even small amounts of radioactive substances are hazardous, particularly when inhaled. Rajewski [23] emphasized that only one-fifth as much radium is required to produce the same effects when inhaled or injected intravenously as when taken by mouth. Likewise, Aub [22] stated that lead inhaled is more toxic than that taken by mouth. Stevens,[24] in reporting a case of radium poisoning following intravenous injection of radium chloride for Hodgkin's disease, pointed out that the percentage of permanent fixation of radium in the tissues is vastly greater when the chemical is injected directly into the blood stream than when it is taken orally. From a clinical standpoint, lead and radium poisoning are also similar. Lead [22] and radium [24] have a predilection for lodgment in the bones. According to St. George and co-workers,[24d] the necrosis, osteitis and rarefaction of bone in radium poisoning is due to the bombardment of the bones by radium rays. A hypochromic type of anemia is commonly associated with radium [24c] and lead intoxication. These metals [25] conspicuously involve the bones of the jaw, which are especially vulnerable to infection. An occasional case of lead poisoning is seen in which the toxic manifestations of lead seem much out of proportion to the amount of lead taken in, and it is likely that radioactive substances when present are important in enhancing the toxic effect of lead. Inasmuch as commercial lead contains varying amounts of radioactive substances, usually radium D, it likewise should be considered as a possible inciting factor in bronchiogenic carcinoma when such dust and fumes are inhaled over long periods. Although this source possibly does not prevail widely, it conceivably is a definite hazard.

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21. Crawford, B. L.; Stewart, H. L.; Willoughby, C. E., and Smith, F. L.: Am. J. Cancer **33**:401, 1938.

22. Aub, J. C.: J. A. M. A. **104**:87, 1935.

23. Rajewski, B.: Radiology **32**:57, 1939.

24. (*a*) Stevens, R. H.: Radiology **39**:39, 1942. (*b*) Gettler, A. O., and Norris, C.: J. A. M. A. **100**:400, 1933. (*c*) Martland, H. S.; Colon, P., and Knef, J. P.: ibid. **85**:1769, 1925. (*d*) St. George, A. V.; Gettler, A. O., and Muller, R. H.: Arch. Path. **7**:397, 1929.

25. Aub,[22] Rajewski,[23] Stevens,[24a] Gettler and Norris,[24b] Martland and others.[24c]

REPORT OF TWO CASES

CASE 1.—A white man aged 57, when admitted to the Edward W. Sparrow Hospital, Lansing, Mich., Jan. 6, 1942, complained of general weakness progressive in character, left foot drop, diplopia, great fatigue on exertion, insomnia and anorexia. He gave a history of having had lead poisoning on two previous occasions, nine and five years ago. Both attacks were associated with intestinal colic and were treated by his physician. The patient had been employed as a linotype operator steadily for thirty-five years. During his work he was exposed to the fumes of molten metal from the linotype machines over a long period. No history of cancer in the family was elicited.

A general examination revealed an overweight white man. There was palsy of the right eye with inability to rotate the eye outward. The other cranial nerves were normal. All of the deep reflexes were slightly increased. The Babinski, Oppenheim and Chaddock signs were negative. The left knee jerk was definitely increased. No ankle clonus was found. The left leg was weaker than the right with a suggestion of a left foot drop. The gums were bluish, and the teeth were carious. The abdomen, the heart and the lungs were essentially normal.

Roentgen examination of the chest revealed an abnormal density between the upper and middle lobes of the right lung. The remainder of the upper lobe of the right lung had a mottled appearance. All other portions of the lung were apparently clear. In addition, a mass was seen extending from the superior mediastinum into the right side of the chest.

The blood on admission contained hemoglobin 81.5 per cent, erythrocytes 4,700,000 and leukocytes 16,000, with 70 per cent neutrophils, 21 per cent lymphocytes, 4 per cent eosinophils and 5 per cent monocytes. No evidence of basophilic stippling was found, but reticulocytes, stippling of erythrocytes and a severe grade of secondary anemia had been observed by the attending physician on several occasions before admission. January 29, the urine revealed 0.15 mg. of lead per thousand cubic centimeters.

The clinical diagnosis was chronic lead poisoning and carcinoma of the lung with metastasis to the mediastinum.

The patient died March 20. The autopsy revealed a neoplasm of the right lung arising in the main bronchus to the middle and upper lobes of the right lung, diagnosed as bronchiogenic carcinoma. Microscopic examination of the tumor of the right lung showed it to be composed of small, polyhedral, deeply staining, poorly differentiated cells. In several areas the neoplasm extended downward from the mucosa of the large bronchus. It extended internal and external to the cartilage plates with metastases to the regional lymph nodes. Gangrenous necrosis of the infarcted area peripheral to the tumor mass of the right lung was seen. Marked general fibrosis and connective tissue hyalinization of the alveolar walls of both lungs were observed. Marked connective tissue hyalinization with calcification was found in the region of the primary site and about some of the metastatic nodules of the right lung. The pleural surfaces showed marked fibrosis and thickening. Acute purulent bronchitis and bronchopneumonia were encountered, which involved chiefly the posterior one third of both lungs. Metastases were found in the peribronchial, peritracheal and mediastinal lymph nodes and in the liver.

CASE 2.—A white man aged 64, when admitted to the Mercy Hospital, Jackson, Mich., Sept. 15, 1941, complained of a productive cough, dyspnea increasing in severity, progressive weakness, night sweats and pain in the left side of the chest. The present illness began about four months prior to admission. The patient had

been in apparently good health up to the present illness and had not consulted a physician. He gave a history of working in an industrial plant as a metal polisher for over ten years in his early career. He worked as a painter during the latter part of his life. No family history of cancer was elicited.

The patient was a well developed and fairly well nourished elderly white man. Diminished breath sounds were present over the left side of the chest, associated with dry and moist rales. The left side of the chest below the inferior angle of the scapula was dull to percussion. A more thorough examination was not permitted because of the grave state of the patient.

The urine showed nothing remarkable. The blood on admission contained hemoglobin 72 per cent, erythrocytes 3,900,000 and leukocytes 17,000, with 86 per cent neutrophils, 12 per cent lymphocytes, 1 per cent eosinophils and 1 per cent monocytes. Nucleated red cells and marked polychromatophilia were seen. The clinical diagnosis was probable carcinoma of the left lung with pleural effusion on the left. No tubercle bacilli were found in either the sputum or the aspirated pleural fluid.

The patient died one week after admission. The autopsy disclosed advanced bronchiogenic carcinoma of the left lung, miliary in type, extensively infiltrating both lungs and pleura. Microscopic examination of the lungs revealed a diffusely infiltrating neoplasm composed of small, irregular shaped, poorly differentiated cells. The primary site appeared to be located in the mucosa and wall of the large bronchus to the upper and lower lobes of the left lung because of the extensive degenerative changes in this region, such as connective tissue hyalinization, cavity formation and calcification. No evidence of tuberculosis was seen. The remainder of the left lung and the entire right lung showed extensive fibrosis of the alveolar walls with well marked connective tissue hyalinization. Extensive fibrous pleuritis of the lungs was encountered, which was extensively infiltrated by the neoplasm. Acute purulent bronchitis and bronchopneumonia were found involving the posterior one third of both lungs.

COMMENT

In the 2 cases now presented, exposure to lead continued for many years. An important point, often emphasized, is that carcinoma may not manifest itself until many years after exposure to irritating dusts, which is borne out by these cases. Both the patients were in the typical age group. The first had recurrent attacks of lead intoxication and, in addition, an amount of lead in the urine indicating that lead was actually taken into the body. At autopsy chronic fibroid pneumonitis was found in both cases, suggesting chronic irritation in some form.

Caution should be exercised before concluding that the inhalation of lead has been an inciting factor in bronchiogenic carcinoma. Each case should be carefully studied individually and interpreted absolutely on its own merits. The mere fact that a man worked in a lead industry does not necessarily mean that he has been exposed to the hazards of lead, but a long history of definite exposure to commercial lead dusts and fumes would be significant.

Several cases of lead poisoning have been reported in the establishment where the first patient was employed. In such cases a thorough investigation should be made to determine whether the establishment has

been taking adequate precautions against the hazards of lead. Outbreaks of epidemics of lead poisoning over a long period in the same establishment mean that proper precautions have not been taken. Tests of the atmosphere about the molten pots of lead on the linotype machines where the first patient was employed showed, on one occasion, 16.1 mg. of lead per 10.0 cubic meters of air. Although this sample did not represent the operator's exposure, it did indicate a potential hazard because of an inadequate ventilating system. Moreover, examination with the Geiger counter of three lead slugs from the establishment showed that one slug was slightly radioactive. Although this finding is not important from the standpoint of quantity, it is of interest as it demonstrates a convenient means of checking commercial lead for radioactivity. When tissues are removed, particularly bone, they can be quickly checked for radioactivity by means of the Geiger counter. Regions of the body can also be readily examined for radioactivity by this instrument. Evans [26] described a method in the use of the Geiger counter which is of great practical importance in medicine. By measuring the amount of gamma radiation, he can estimate the amount of radium present. When only traces of radioactive substances are present, it may be necessary to do a quantitative chemical analysis, because small amounts may not be detected by this instrument.

SUMMARY

Carcinoma of the lung in lead workers has been recorded in the literature. The Schneeberg cases are the only conclusive group of cases of bronchiogenic carcinoma associated with the inhalation of radioactive substances. Because commercial lead is by far the most radioactive of all the common metals, owing to the presence of radioactive elements, particularly radium D, it may be a possible factor in the production of bronchiogenic carcinoma when lead dusts and lead fumes are inhaled.

Two cases of bronchiogenic carcinoma are reported. Both patients gave a long history of occupational contact with commercial lead. The first gave a protracted history of exposure and intoxication. Both showed chronic fibroid pneumonitis.

26. Evans, R. D.: Am. J. Roentgenol. **37**:368, 1937.

MORPHOLOGIC CHANGES IN THE RAT'S ADRENAL CORTEX UNDER VARIOUS EXPERIMENTAL CONDITIONS

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It is well recognized today that disturbances in electrolyte, carbohydrate and protein metabolism influence the activity of the adrenal gland. Considerable information is available concerning the interdependence between the adrenal and the other endocrine glands, particularly the gonads and the pituitary, increased and decreased function of the latter profoundly affecting adrenal function. This study, undertaken with the view first expressed by Virchow that every physiologic process has its anatomic counterpart, is concerned with the morphologic changes in the rat's adrenal cortex associated with various endocrine and metabolic disturbances. These include hypophysectomy, castration, variations in the potassium and the protein content of the diet, inanition and administration of desoxycorticosterone acetate and stilbestrol. Knowledge of the life cycle of the cortical cell (Zwemer [1]) in its migration from the zona glomerulosa through the zona fasciculata to die in the zona reticularis and the more recently available histochemical methods which identify the cortical steroid hormones and cholesterol respectively (Bennett [2]) lend added significance to the histologic changes and alterations in lipoid pattern observed in the adrenal cortex under various experimental conditions, thereby opening the way to physiologic interpretations.

MATERIALS AND METHODS

The adrenals examined were obtained in large part from rats being studied for other purposes by various investigators working in the laboratories of the Yale School of Medicine. Drs. D. C. Darrow and S. H. Durlacher supplied rats maintained on a low potassium diet and others given desoxycorticosterone acetate; Dr. J. A. Russell performed the hypophysectomies; Drs. F. Engel and J. Tepperman provided rats maintained on a high protein diet, and Miss Edith Fry, stilbestrol-treated rats. Male and female rats of Wistar, Yale and Sprague-Dawley strains, weighing approximately 200 Gm., were studied. The adrenals with the surrounding fat were carefully removed at autopsy and immediately placed in 4 per cent solution of formaldehyde. After two days, following the removal of the periadrenal tissues, the glands were dried on paper toweling and weighed

This study was aided by a grant from the Commonwealth Fund.
From the Laboratory of Pathology of the Yale University School of Medicine.

1. Zwemer, R. L.: Am. J. Path. **12**:107, 1936.
2. Bennett, H. S.: Am. J. Anat. **67**:151, 1940.

on a chainomatic balance to the nearest milligram. The weights of the glands are always expressed as grams per kilogram of body weight. Paraffin sections were stained with hematoxylin and eosin, and frozen sections, cut at 10 to 15 microns, were stained with sudan IV. The phenylhydrazine and digitonin technics (as employed by Bennett [2]) were used on selected adrenals to demonstrate corticosteroids and cholesterol, respectively.

THE NORMAL ADRENAL CORTEX

To determine the significance of an adrenal weight one must necessarily consider the weight of the animal. Furthermore, in those experimental conditions in which the animal loses weight, the ratio of the adrenal weight to the original body weight as well as to the final body weight should be considered. False conclusions are easily drawn from absolute adrenal weights if the ratio of each of the latter to the body weight is not taken into account. Using rats from the Yale and Wistar strains, Cole and Harned [3] found straight line relationships between adrenal and body weights in males and females whose body weight varied from 100 to 320 Gm. That the female adrenal is heavier is well known. Andersen and Kennedy [4] showed that the adrenals of females are slightly larger in estrus. The weights of the adrenals of the normal male and female rats of the Wistar strain are shown in table 1.

Microscopic examination of the normal rat adrenal reveals the zona glomerulosa to be several cells thick and laden with lipoid (fig. 1). A thin band of fat-free polygonal cells comprises the junction of the zona glomerulosa and the zona fasciculata ("clear zone"). The cells of the zona fasciculata exhibit an orderly columnar arrangement. Their cytoplasm is foamy and contains abundant lipoid in the form of uniformly moderate-sized droplets. The outer part of the zona fasciculata is much richer in lipoid than the inner. The former corresponds to Bennett's "secretory layer," while the latter is the "discharging layer." The zona reticularis in the normal rat adrenal is poorly defined, consisting of a narrow band of cells in various stages of degeneration. No histologic sex differences can be ascertained aside from the wider cortex in the female.

Employing phenylhydrazine and digitonin on frozen sections, one sees that the cortical steroids and the cholesterol are most concentrated in the outer half of the zona fasciculata with somewhat less in the zona glomerulosa. In general it can be said from histochemical studies of adrenals of rats as well as of those of man and dogs [5] that the

3. Cole, V. V., and Harned, B. K.: Endocrinology **30**:146, 1942.

4. Andersen, D. H., and Kennedy, H. S.: J. Physiol. **76**:247, 1932.

5. Sarason, E. L.: (*a*) A Morphological Study of the Adrenal Cortex in Systemic Disease, to be published; (*b*) unpublished data.

distribution of the ketosteroids and of the cholesterol corresponds closely to the intensity of the sudanophilic material. For this reason histochemical studies were not employed routinely in the present study, as it was believed that the all-inclusive sudan stain served the purpose adequately.

DESOXYCORTICOSTERONE ACETATE

Crystalline desoxycorticosterone acetate (abbreviated in tables to DOCA)[6] was dissolved in warm 95 per cent alcohol and added to physiologic solution of sodium chloride so that each cubic centimeter contained 2 mg. of finely suspended precipitate in 7 per cent alcohol. Two milligrams of the drug was administered subcutaneously daily for one month to 19 male and 10 female rats of the Wistar strain. The weights of the glands as compared with normal weights are presented in table 1.

TABLE 1.—*Effect of Desoxycorticosterone on the Weight of the Rat Adrenals*

Group	Sex	Rats *	Adrenal Weight, Gm. per Kg.	p †
Normal	M	8	0.1449 ± 0.0049	<0.01
DOCA-treated	M	19	0.0997 ± 0.0064	
Normal	F	20	0.2382 ± 0.00527	>0.05
DOCA-treated	F	10	0.2260 ± 0.0061	

* The rats were given 2 mg. of desoxycorticosterone acetate daily for one month.

† According to R. A. Fisher (Statistical Methods for Research Workers, London, Oliver & Boyd, 1941, pp. 120-125), a value of p greater than ($>$) 0.05 indicates no significant difference between the means; a value of p less than ($<$) 0.05 but greater than 0.01 indicates a significant difference; a value of p less than 0.01 indicates a highly significant difference.

Following daily administration of 2 mg. of desoxycorticosterone acetate for one month, there occurred considerable atrophy of the male adrenal but no significant change in the female adrenal. One is struck by the variance of results reported in the literature concerning the effects of cortical hormones. Wells and Kendall[7] reported adrenal atrophy occurring eight days after administration of corticosterone, but no atrophy after 10 mg. of desoxycorticosterone suspended in 0.7 per cent solution of sodium chloride had been injected subcutaneously every third day for nine days. Selye and Dosne[8] observed slight cortical atrophy following daily administration of 2 mg. of desoxycorticosterone acetate for twenty days, and more pronounced atrophy after 10 mg. had been given daily for a similar period. Selye[9] stated that the atrophy follow-

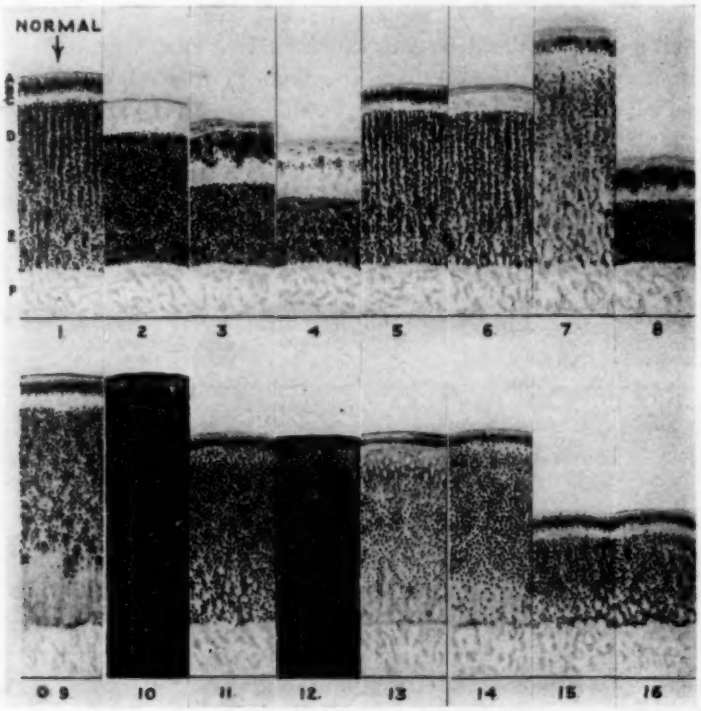
6. The crystalline desoxycorticosterone was generously supplied by the Ciba Company.

7. Wells, B. B., and Kendall, E. C.: Proc. Staff Meet., Mayo Clin. **15**: 133, 1940.

8. Selye, H., and Dosne, C.: Proc. Soc. Exper. Biol. & Med. **44**:165, 1940.

9. Selye, H.: Canad. M. A. J. **42**:113, 1940.

ing administration of this compound is more pronounced in the female. As may be seen from table 1, the comparatively small dose of 2 mg. of desoxycorticosterone acetate causes significant atrophy only in the male. Carnes and his co-workers [10] observed more striking atrophy in the male than in the female following daily administration of 5 mg. of this substance for one month. Ingle [11] found it necessary to administer larger amounts of an extract of adrenal cortex to females than to males in order to produce a loss in the weight of the adrenal glands. It is known that castration of the female may result in atrophy of the



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adrenal glands. Administration of estrogen causes hypertrophy of the gland [12] while that of testosterone has the opposite effect. It would be expected, therefore, that the female adrenal would be more resistant to atrophy.

Ingle and Kendall [13] found that the administration of large amounts of extract of adrenal cortex over a period of seven days caused atrophy

10. Carnes, W. H.; Ragan, C.; Ferrebee, J. W., and O'Neill, J.: Endocrinology **29**:144, 1941.

11. Ingle, D. J.: Endocrinology **24**:194, 1939.

12. Bourne, G., and Zuckerman, S.: J. Endocrinol. **2**:283, 1941.

13. Ingle, D. J., and Kendall, E. C.: Science **86**:245, 1937.

Fig. 1.—Normal rat adrenal. The black dots represent sudanophilic material. *A* indicates thin acellular fat-free capsule; *B*, zona glomerulosa; *C*, "clear zone" of the outer part of the zona fasciculata; *D*, outer part of the zona fasciculata; *E*, inner part of the zona fasciculata; *F*, medulla.

Fig. 2.—Rat B1, treated with 2 mg. of desoxycorticosterone acetate daily for one month. Note the atrophy of the cortex and the depletion of lipoid in the zona glomerulosa.

Fig. 3.—Rat H16, a hypophysectomized rat maintained on a normal diet (one month after hypophysectomy). Note the thickened cellular capsule with a few lipoid-containing cells, the widened zona glomerulosa, rich in lipoid, and the prominent clear zone. The sudanophilic material of the shrunken zone fasciculata is in the form of large droplets.

Fig. 4.—Rat H12, a hypophysectomized rat treated with 2 mg. of desoxycorticosterone acetate daily for one month. Note the further atrophy of the cortex with depletion of lipoid of the zona glomerulosa.

Fig. 5.—Rat B6, an ovariectomized rat. Note the relatively normal appearance of the cortex.

Fig. 6.—Rat B11, an ovariectomized rat treated with 2 mg. of desoxycorticosterone acetate daily for one month. Note the zona glomerulosa depleted of lipoid.

Fig. 7.—Rat S18, a female, starved for seven days. Note the hypertrophy of the cortex and the general depletion of lipoid. Pseudoacini are seen in the inner part of the zona fasciculata.

Fig. 8.—Rat H3, a hypophysectomized animal, suffering from chronic inanition for one month. Note the striking atrophy of the inner part of the zona fasciculata with the lipoid present in the form of unusually large droplets.

Fig. 9.—Rat HP1, a male fed a high protein–low carbohydrate diet for one month. Note the hypertrophy of the cortex with depletion of the lipoid of the inner part of the cortex. Compare with the control (fig. 11).

Fig. 10.—Rat HP1, same as in figure 9. Frozen section treated with digitonin and examined with the polariscope. The white dots represent birefringent cholesterol crystals. Note the general depletion of the latter in the zona fasciculata with the increased amount in the zona glomerulosa. Compare with the control (fig. 12).

Fig. 11.—Rat CT1—Litter mate of rat HP1 fed the normal chow diet. Contrast with figure 9.

Fig. 12.—Rat CT1.—This is a digitonin preparation of an adrenal of the animal fed the chow diet. Note the general distribution of the birefringent cholesterol crystals through the entire cortex. Compare with figure 10.

Fig. 13.—Rat ST2, a starved male rat given an injection of stilbestrol. Note the general depletion of lipoid associated with the presence of vacuolated non-lipoid-containing cells in the outer part of the zona fasciculata. Compare with figure 14.

Fig. 14.—Rat C2, a male rat starved for a period similar to rat ST2 but not treated with stilbestrol. Note the relatively normal-appearing cortex as compared with figure 13.

Fig. 15.—Rat SH2, a hypophysectomized, starved rat treated with stilbestrol. The animal was put to death fifty-four hours after hypophysectomy. Note the atrophy of the cortex with but little change in the lipoid pattern.

Fig. 16.—Rat FH4, a hypophysectomized starved rat not treated with stilbestrol. Note that the cortex appears similar to that of the stilbestrol-treated hypophysectomized animal (fig. 15).

of the adrenals of male rats. Ingle, Higgins and Kendall later reported [14a] that compound A and corticosterone also produced adrenal atrophy. Ingle [14b] demonstrated that simultaneous administration of an adrenotropic fraction of the pituitary gland prevents the atrophy of the adrenal that ordinarily follows treatment with extract of adrenal cortex alone. It was therefore suggested by Ingle that the adrenotropic activity of the pituitary gland is suppressed by excessive extract of adrenal cortex and that in this manner adrenal atrophy is produced. It is difficult to subscribe to this hypothesis in view of the radically different histologic appearance of the adrenal after hypophysectomy and administration of cortical extract (figs. 2 and 3). There was observed in the rats (both males and females) treated with desoxycorticosterone acetate a reduction in the size of the zona glomerulosa associated with a disappearance of the sudanophilic lipoid, cholesterol and cortical steroids. Similar depletion of the lipoids was found by Flexner and Grollman [15] (employing the osmic acid technic) after administration of extract of adrenal cortex and by Carnes and associates,[10] who treated rats with desoxycorticosterone acetate.

The depletion in the glomerulosa cell lipoid following administration of excess extract of adrenal cortex was the basis in part for the statement of Flexner and Grollman [15] that depression of adrenal activity is associated with loss of cortical lipoid. Dosne and Dalton [16] and Selye [17] took issue with this conclusion. They expressed the belief that the amount of cortical lipoid demonstrated by either sudan stain or osmic acid definitely decreases as the adrenal enlarges with increased activity. This view is borne out in my experience.[5] I have observed in human cases of overwhelming infection and cachexia markedly enlarged adrenals depleted of lipoid. Severe depletion has been noted in the adrenals of rats exposed to high altitudes [18]—conditions which have been shown by Evans [19] and Thorn and co-workers [20] definitely to activate these glands. It would appear, therefore, that the stimulated adrenal cortex often becomes depleted of lipoid. This might be interpreted as evidence of rapid discharge and minimal storage of cortical hormone during periods of heightened adrenal activity in an

14. (*a*) Ingle, D. J.; Higgins, J. M., and Kendall, E. C.: Anat. Rec. **71**:363, 1938. (*b*) Ingle, D. J.: Am. J. Physiol. **124**:369, 1938.

15. Flexner, L. B., and Grollman, A.: Anat. Rec. **75**:207, 1939.

16. Dosne, C., and Dalton, A. J.: Anat. Rec. **80**:211, 1941.

17. Selye, H.: Endocrinology **21**:169, 1937.

18. Sarason, E. L.: To be published.

19. Evans, G. T.: (*a*) J. Biol. Chem. **105**:34, 1934; (*b*) Am. J. Physiol. **114**:297, 1936.

20. Lewis, R. A.; Thorn, G. W.; Koepf, G. F., and Dorrance, S. D.: J. Clin. Investigation **21**:33, 1942.

effort to meet the demands of the body for increased amounts of the vital hormone. It cannot be denied that in general an endocrine organ is rendered inactive by administration of excessive amounts of its hormone. Such must be the case with the adrenal glands after administration of desoxycorticosterone. It must be borne in mind, therefore, that under certain conditions the absence of cortical lipoid may be associated with either activity or rest of the gland.

VARIATIONS OF POTASSIUM IN THE DIET

Male and female rats of Sprague-Dawley and Wistar strains were maintained for one month on (*a*) a diet low in potassium, (*b*) a low potassium diet plus drinking water containing potassium chloride in the concentration of 1.5 per cent and (*c*) regular Purina fox chow plus the 1.5 per cent potassium chloride drinking water. The latter combination represented the high potassium diet. The synthetic low potassium diet as used by Durlacher, Darrow and Winternitz [21] contained 1.6 millimols of potassium per hundred grams, while the potassium content of the Purina chow was 15.5 millimols per hundred grams and that of the synthetic diet with added potassium chloride 16.6 millimols per hundred grams. The adrenal weights are presented in table 2.

TABLE 2.—*Effect of Variations of the Potassium Content of the Diet on the Weight of Rat Adrenals*

Diet	Sex	Rats	Adrenal Weight, Gm. per Kg.	p
Purina chow	M	8	0.1449 ± 0.0049	>0.05
Chow + KCl	M	5	0.1559 ± 0.0085	
Low K	M	10	0.1328 ± 0.0028	>0.05
Low K + KCl	M	8	0.1382 ± 0.0061	
Low K	F	4	0.2560 ± 0.0187	>0.05
Chow + KCl	F	5	0.2688 ± 0.0121	

From the data in table 2 it is seen that the adrenals of these rats exhibited no significant change in weight whether the animals were fed a diet low or a diet rich in potassium. Microscopic examination of these glands revealed no apparent alteration in the lipoid pattern of the cortex. Ingle and Kendall [22] likewise observed no change in the size of the adrenal glands of animals fed different amounts of sodium and potassium.

The experiments described shed light on the nature of the effect of desoxycorticosterone acetate on the adrenals. Animals fed a low potassium diet or given injections of this compound exhibit a similar

21. Durlacher, S. H.; Darrow, D. C., and Winternitz, M. C.: Am. J. Physiol. **136**:346, 1942.

22. Ingle, D. J., and Kendall, E. G.: Am. J. Physiol. **122**:585, 1938.

loss of muscle potassium accompanied by low serum potassium values (Heppel [23]; Miller and Darrow [24]). Whereas desoxycorticosterone acetate (2 mg. daily for one month) produced atrophy of the adrenal, this was not observed in animals fed a low potassium diet. Furthermore, the shrinkage of the cells of the zona glomerulosa associated with the depletion of lipoid seen following administration of the drug was not observed in the low potassium group. It is well recognized that the adrenal and the kidney are intimately concerned with potassium metabolism. It appears that whereas renal hypertrophy results from lowering of the muscle and serum potassium by either dietary methods or by administration of desoxycorticosterone acetate,[21] the adrenal atrophy

TABLE 3.—*Effect of Hypophysectomy on the Weight of the Adrenals of the Female Rat*

Group	Rats	Adrenal Weight, Gm. per Kg. of Initial Body Weight	p
Normal	20	0.2382 ± 0.0052	<0.01
Hypophysectomized	4	0.0929 ± 0.0028	

TABLE 4.—*Effect of a Low Potassium Diet, of Desoxycorticosterone and of Chronic Inanition on the Weight of the Adrenals of the Hypophysectomized Female Rat*

Group	Rats	Adrenal Weight, Gm. per Kg. of Initial Body Weight	p
Normal diet	4	0.0929 ± 0.0028	>0.05
Low K diet	4	0.1024 ± 0.0030	
DOCA	5	0.0797 ± 0.0035	<0.05; >0.01
Inanition	3	0.0651 ± 0.0056	<0.01

produced by the latter is not dependent directly on a deficiency of body potassium.

HYPOPHYSECTOMY

The adrenals of 16 female and 4 male rats were examined one month and two days, respectively, after hypophysectomy. Some of the hypophysectomized animals were maintained on a low potassium diet, and others were given 2 mg. of desoxycorticosterone acetate daily during the postoperative month. The weights of the adrenal glands are presented in tables 3 and 4.

In hypophysectomized female rats maintained on a normal diet a considerable degree of atrophy of the adrenal cortex was found one month after operation. The usual acellular capsule had been trans-

23. Heppel, L. A.: Am. J. Physiol. **127**:385, 1939.

24. Miller, H. C., and Darrow, D. C.: Am. J. Physiol. **132**:801, 1941.

formed into a band rich in oval-shaped cells, some of which contained sudanophilic material (fig. 3). The zona glomerulosa was intact, if not a little wider than usual, and contained abundant lipoid. Digitonin preparations revealed that the sudanophilic zona glomerulosa was laden with cholesterol. The fat-free clear zone of the outer part of the zona fasciculata was very prominent. The cells comprising the outer part of this zone lacked their usual regular columnar arrangement, and their cytoplasm appeared vacuolated instead of foamy. The sudan stain of these cells was in the form of large droplets of varying size, rather than the usual uniformly small droplets. The inner part of the zona fasciculata was sparse in cells, and their cytoplasm contained abundant yellow-green pigment. Pyknotic and extruded nuclei were frequent in the zona reticularis, as well as marked congestion and occasional hemorrhage.

The adrenals of hypophysectomized rats fed a low potassium diet differed neither grossly nor microscopically from those of hypophysectomized animals given the regular diet. Further significant reduction in adrenal size was seen when the hypophysectomy was followed by the administration of desoxycorticosterone acetate (2 mg. daily for thirty days). Histologically, as in the intact animal, the adrenals of the desoxycorticosterone-treated hypophysectomized rat exhibited marked depletion of the lipoid of the zona glomerulosa (fig. 4).

The striking adrenal atrophy of hypophysectomized rats suffering from chronic inanition will be discussed in later paragraphs in connection with the general problem of inanition.

The adrenals of 4 hypophysectomized male rats (Yale strain) that had been subjected to a fifty-four hour postoperative fast were found to have already undergone atrophy (table 8). (Two of these rats had received a single injection of stilbestrol. As in the intact animal, in the hypophysectomized animal the stilbestrol did not affect the size of the adrenals.) Although atrophy had resulted in so short a time following hypophysectomy, the histologic structure was not perceptibly altered except for shrinkage of the cortex (fig. 16).

In 1930 Smith [25] reported marked adrenal atrophy following ablation of the pituitary, with restoration of the adrenals to normal size following replacement therapy. Cutuly [26] has shown conclusively that the atrophy is exclusively cortical. Smith noted the retrogression of the cortex to be rapid, the adrenals in 1 animal killed six days after hypophysectomy losing half of their weight. From the data in table 8 it is seen that after hypophysectomy followed by a fifty-four hour fast the adrenals are decreased one third in weight. Crooke and Gilmour [27]

25. Smith, P. E.: Am. J. Anat. **45**:205, 1930.
26. Cutuly, E.: Anat. Rec. **66**:119, 1936.
27. Crooke, A. C., and Gilmour, J. R.: J. Path. & Bact. **47**:525, 1938.

reported a decrease in adrenal weight from a normal value of about 19 mg. to 15 mg. and 12 mg., two and four days, respectively, after hypophysectomy. Perla [28] described degenerative changes in the zona reticularis as early as four days after hypophysectomy. Crooke and Gilmour [27] likewise found that loss of cortical cells and degenerative changes did not appear until four days after hypophysectomy. They observed that the cortical atrophy was confined to the inner zone, the outer cortical zone being increased in depth. Some of the pigment present in the inner cortical cells was shown by these authors to be iron positive, the remainder apparently being lipofuscin. These findings are confirmed in the present study.

Whereas Smith [25] observed abundant lipoid in the outer cortex with depletion in the inner cortex, the histologic picture seen in the present study can best be described as a "bull's eye"—the widened zona glomerulosa and the atrophied zona fasciculata filled with lipoid separated by a wide clear zone free of fat. A morphologic interpretation of the histologic changes in the cortex following hypophysectomy is possible on the basis of the theory (Zwemer [1]; Bennett [2]) postulating that the cortical cells originate from the capsule and migrate through the cortex to die in its inner portion; the proliferation of primitive capsular cells (some containing lipoid) and of young cells of the zona glomerulosa may be an attempt to replace the aging inner cortical cells which suffer premature death following hypophysectomy. Leblond and Nelson [29] observed persistence of lipoid in the atrophic inner part of the cortex following hypophysectomy. In view of the work of Selye [17] and of Houssay and associates [30] showing that very fine powder-like sudanophilic material is indicative of adrenal activation, it should be pointed out that the atrophic inactive inner portion of the cortex is the site of large droplets of sudan. Whereas after hypophysectomy the derangement of electrolyte and water metabolism is only slight, as compared with the profound disturbances in carbohydrate metabolism, it has been stated by Swann [31] that "presumably, the portions of the cortex only slightly affected by hypophysectomy, i. e., primarily the glomerulosa layer, secrete the 'salt and water' hormone. . . . Since hypophysectomy leads to degeneration of the internal layers of the cortex, it seems plausible to ascribe to them the adrenal secretions not produced after hypophysectomy, i. e., the steroids responsible for the effects on sugar metabolism."

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28. Perla, D.: Proc. Soc. Exper. Biol. & Med. **32**:655, 1935.

29. Leblond, C. P., and Nelson, W. V.: Compt. rend. Soc. de biol. **124**:9, 1937.

30. Houssay, B. A.; Busotti, H.; Mazzacco, P., and Sammartino, R.: Compt. rend. Soc. de biol. **144**:739, 1933.

31. Swann, H. G.: Physiol. Rev. **20**:493, 1940.

Ingle and co-workers[14a] stated: "The extent of atrophy of the glands of animals treated with cortin resembled that which occurs in totally hypophysectomized animals." Because the administration of a pituitary extract containing the adrenotropic hormone prevents the adrenal atrophy resulting from the administration of extract of adrenal cortex, it has been argued by Ingle[14b] that the atrophy is the result of pituitary depression following the administration of the extract of adrenal cortex. Were this so, one would expect the histologic changes in the adrenal following hypophysectomy to be similar to those following administration of desoxycorticosterone acetate. This is not the case. Whereas the adrenal of the rat treated with desoxycorticosterone acetate has a lipoid-poor zona glomerulosa and a normal-appearing zona fasciculata, the gland of the hypophysectomized animal has a widened zona glomerulosa, abundant in lipoid, associated with a zona fasciculata showing marked shrinkage. The even more marked adrenal atrophy and

TABLE 5.—*Effect of Acute Inanition on the Weight of Rat Adrenals*

Starvation Period	Sex	Rats	Loss of Body Weight	Adrenal Weight: Gm. per Kg. Initial Body Weight	Adrenal Weight: Gm. per Kg. Final Body Weight	p
Fed controls	M	8	0	0.1449 ± 0.0049		
One day	M	3	12%	0.1511 ± 0.0051	0.1716	>0.05
Two days	M	3	15%	0.1640 ± 0.0042	0.1939	<0.05; >0.01
Seven days	M	6	25-30%	0.1576 ± 0.0068	0.2235	<0.05; >0.01
Fed controls	F	20	0	0.2383 ± 0.0052		
Seven days	F	3	25-28%	0.2809 ± 0.0351	0.3966	<0.01

depletion of lipoid of the zona glomerulosa of the hypophysectomized rat treated with desoxycorticosterone acetate as compared with the treated intact animal is more evidence against the theory that the atrophy following administration of desoxycorticosterone acetate is mediated through the pituitary.

INANITION

Male and female rats of the Wistar strain were subjected to acute inanition for a period of one to seven days, during which time they received drinking water but not food. The weights of the adrenal glands are presented in table 5.

During acute inanition the adrenals, in contrast to the other viscera, lost no weight; on the contrary, they gained a little and thereby increased their relative and absolute percentage weights. The data in table 5 confirm the findings of Jackson,[32] who observed that the adrenals of male rats during acute inanition gained 1.5 per cent in weight. This increase was not regarded by Jackson as significant. A statistical analy-

32. Jackson, C. M.: Am. J. Anat. **25**:221, 1919.

sis of the data in table 5 reveals that the slight increase in the adrenal weight of male rats subjected to one day of starvation is not significant, while the increases seen following two and seven days' starvation are significant. Even more significant adrenal hypertrophy was observed in females starved seven days. This sex difference is apparent from the data of Mulinos and Pomerantz [33] if the original body weights of the animals are taken into account. From their table it appears that the average adrenal weight of the male rats increased from 28 to 34 mg. after seven days of starvation. They stated that the starved animals weighed 277 Gm. before starvation, in contrast to the fully fed controls, weighing 200 Gm. A definite linear relationship between adrenal and body weight is known to exist. Hence, if the difference between the initial weights of the starved animals and those of the controls is taken into account, one cannot admit that any significant hypertrophy of the male adrenals has occurred. These authors observed an increase in the weight of the female adrenals during acute inanition comparable to that noted in table 5.

Microscopic examination reveals no constant lipoid pattern of the adrenals during acute inanition. The glands of about half the animals that had fasted for seven days showed varying degrees of depletion of the lipoid of the zona fasciculata, with sudanophilic material persisting in the zona glomerulosa (fig. 7). A more uniform distribution of fine powder-like sudanophilic material was noted throughout the cortex in the other animals, the clear zone and the inner part of the zona fasciculata possessing more lipoid than usual. Similarly varying patterns of lipoid distribution were described by Jackson.[32] Whitehead [34a] recently reported a striking species difference between guinea pigs and rabbits during acute inanition. Whereas the adrenals of fasting rabbits show no alteration in the amount or the distribution of cortical fat, the glands of guinea pigs show a varying lipoid pattern depending on the duration of the inanition. In general it appears that Whitehead's observations on the guinea pig correspond closely to the findings presented in this study, i. e., increase of inner cortical lipoid during the first few days of starvation with a tendency toward depletion of fat after a week or more of inanition. Dosne and Dalton [16] observed some reduction of the lipoid content of the cortex in the adrenals of rats starved twenty-four hours. The explanation of the variation in lipoid pattern seen in acute inanition is not at hand. The slight increase in cortical lipoid pattern seen after one or two days of fasting may represent increased production of hormone, while the cortical depletion seen in

33. Mulinos, M. G., and Pomerantz, L.: Am. J. Physiol. **132**:368, 1941.

34. Whitehead, R. J.: (*a*) J. Path. & Bact. **54**:169, 1942; (*b*) Brit. J. Exper. Path. **13**:200, 1932.

some animals following more prolonged starvation may be the result of hormone discharge. Formation of pseudoacini at the corticomedullary junction was seen in the lipoid-depleted adrenals of 2 female rats (fig. 7).

Mention should be made of a group of 10 hypophysectomized females which did very poorly postoperatively, 7 dying in the course of the month (the adrenals were not examined). The 3 survivors lost approximately 50 per cent of their body weights during the month. Their food consumption during this period was definitely decreased. The explanation of their slowly progressive decline is not at hand. As may be seen from table 4, the most severe adrenal atrophy, associated with extreme reduction of the inner portion of the cortex, was exhibited by this group of 3 animals (fig. 8). Mulinos and Pomerantz [83] observed that normal pituitary glands implanted into chronically underfed female rats resulted in a gain of weight in the otherwise atrophied adrenal glands. These authors reasoned (after the manner of Ingle [14b]) that the

TABLE 6.—*Effect of a High Protein Diet on the Weight of the Rat Adrenals*

Diet	Sex	Rats	Adrenal Weight, Gm. per Kg.	p
Purina chow	M	18	0.157 ± 0.0087	
High protein	M	18	0.236 ± 0.0115	
				<0.01

adrenal atrophy seen in malnutrition was due in part to the insufficiency of adrenotropic hormone resulting from the physiologic depression of function of the pituitary. The aforementioned data seem to indicate that factors other than depression of the function of the pituitary may effect atrophy of the adrenal in malnutrition.

HIGH PROTEIN DIET

Yale strain rats were fed a diet consisting exclusively of ground lean meat, with a protein content of approximately 45 to 50 per cent, for one month. Other rats of the same strain were fed the basic diet including the fox chow, containing 18 per cent protein. The adrenal weights of these animals are presented in table 6.

The marked increase in size of the adrenals of rats fed a high protein diet is striking. Microscopic examination revealed the hypertrophy to be cortical (fig. 9). The zona glomerulosa was slightly widened by virtue of an increased amount of lipoid in the cells. The cells of the zona fasciculata lacked their usual foamy appearance for the most part, taking on a homogeneously eosinophilic color (hemotoxylin-eosin preparation). They were moderately depleted in lipoid. The clear zone, composed of the outermost fat-free cells of the zona fasciculata, was

increased in width. The digitonin preparations revealed that the zona glomerulosa of the hypertrophied adrenal contained an increased amount of cholesterol and that the zona fasciculata was markedly depleted of birefringent material (fig. 10). The distribution of the latter differs markedly from the uniform distribution of this material through both the zona glomerulosa and the zona fasciculata of the normal adrenal (fig. 12).

Farr [35] described marked adrenal hypertrophy of rabbits which had been fed a diet consisting exclusively of milk and eggs. He noted that the zona fasciculata was widened and the cells vacuolated. Whitehead [34b] reported cortical proliferation in the mouse adrenal after administration of peptone. He stated: "They [the mitoses in the cortex] indicate the response to demand for increased function." Engel and Tepperman,[36] who have studied the metabolism of such meat-fed rats, expressed the belief that the observed adrenal hypertrophy is associated with an increase in the rate of glyconeogenesis, the latter being a consequence of a high protein–low carbohydrate diet. These authors have proposed in a review of adrenal hypertrophy [37] that accelerated protein catabolism is the common denominator of many conditions associated with adrenal hypertrophy. It should be pointed out that the enlarged stimulated adrenals are depleted of lipoid, confirming the view that in general activation of the adrenals is associated with depletion of cortical lipoid. In foregoing paragraphs similar degrees of depletion have been described in association with adrenal hypertrophy following starvation. The same association has been noted by Dosne and Dalton.[16] Enlarged human adrenals depleted of lipoid have been seen in cases of rapidly advancing cachexia associated with cancer.[5a] James and Nelson [38] have reported adrenal hypertrophy in association with increased glyconeogenesis following administration of stilbestrol. Loeser [39] observed enlargement of the glands and loss of lipoid following daily administration of 1 mg. of stilbestrol for three weeks. The adrenals of several rats included in the present study given a single injection of stilbestrol were found to be partially depleted of lipoid although not changed in size (see a later paragraph). From the foregoing data and the work cited it appears that adrenal hypertrophy and depletion of cortical lipoid are characteristic of accelerated protein catabolism as seen in animals fed a high protein diet, subjected to fasting or treated by injection of stilbestrol.

35. Farr, T. H.: Verhandl. d. deutsch. path. Gesellsch. **15**:234, 1912.

36. Engel, F., and Tepperman, S. J.: Unpublished data.

37. Engel, F., and Tepperman, S. J.: Metabolic Determinants of Adrenal Size and Function, to be published.

38. James, R. G., and Nelson, W. O.: Am. J. Physiol. **136**:136, 1942.

39. Loeser, A.: Ztschr. f. d. ges. exper. Med. **105**:430, 1939.

OVARIECTOMY

The adrenals and kidneys of 6 ovariectomized 10 week old Wistar rats were examined one month after operation. Three animals of this group received 2 mg. of desoxycorticosterone acetate daily during the postoperative period. The wet and dry weights of the kidneys were determined. The results are presented in table 7.

Considerable controversy has appeared in the literature (Hashimoto [40]) concerning the effect of ovariectomy on the adrenals. The age of the rat at the time of operation and the length of the postoperative survival are important factors determining the size of the adrenals. In the present series ovariectomy resulted in no significant atrophy of the adrenals one month after operation. Andersen and Kennedy,[41] working with animals of similar age, reported divergent results. Histologi-

TABLE 7.—*Effect of Ovariectomy and Administration of Desoxycorticosterone Acetate on the Weight of the Adrenals and the Weight of the Kidneys in Castrated Female Rats*

Group	Rats	Adrenal Weight, Gm. per Kg.	p	Kidney Weight, Gm. per Kg.	
				Wet	Dry
Normal females	20	0.2382 ± 0.0052		7.62	1.77
Ovariectomized	3	0.2006 ± 0.0285	>0.5	7.02	1.60
Ovariectomized and treated with DOCA	3	0.1990 ± 0.0156	>0.5	9.55	2.05
DOCA-treated normal	10	0.2260 ± 0.0051		9.07*	2.14*

* These weights were reported by Durlacher, Darrow and Winternitz,[21] who have described renal hypertrophy following administration of desoxycorticosterone.

cally, the adrenals of the ovariectomized animals exhibited no alteration in lipoid pattern as compared with the normal (fig. 5).

In view of the resistance of the adrenals of normal female rats to atrophy following the daily administration of 2 mg. of desoxycorticosterone acetate for one month, this compound was given in similar doses to 3 ovariectomized rats. As may be seen in table 7, the adrenals of the animals so treated were not significantly smaller than those of untreated ovariectomized animals although, like the adrenals of normal rats so treated, they showed depletion of the lipoid in the zona glomerulosa (fig. 6).

In view of the reports by Ludden, Krueger and Wright [42] and Durlacher, Darrow and Winternitz [21] concerning the hypertrophy of the kidneys following administration of desoxycorticosterone acetate, the renal weights of the 6 animals in this group were determined. Koren-

40. Hashimoto, E. I.: Anat. Rec. **81**:205, 1941.

41. Andersen, D. H., and Kennedy, H. S.: J. Physiol. **79**:1, 1933.

42. Ludden, J. B.; Krueger, E., and Wright, I. S.: Endocrinology **28**:619, 1941.

chevsky and Ross [43] found that in male rats castration resulted in a decrease in the size of the kidneys while in female rats ovariectomy caused no such change. The latter observation is confirmed by the data in table 7. It will be noted that desoxycorticosterone acetate administered to ovariectomized rats produced adrenal hypertrophy similar to that reported by Durlacher and associates.[21]

STILBESTROL

The adrenals of 4 normal and 4 hypophysectomized male rats (Yale strain) were examined after the rats had undergone a fifty-four hour fast. Two animals in each group had been given a single injection of stilbestrol (5 mg. per hundred grams of body weight) after the first twenty-four hours of fasting. The weights of the glands are presented in table 8.

TABLE 8.—*Effect of Administration of Stilbestrol on the Weight of the Adrenals of Fasting Normal and Hypophysectomized Males*

Group	Rats	Loss of Body Weight	Adrenal Weight, Gm. per Kg.	
			With Initial Body Weight	With Final Body Weight
Normal	2	13%	0.1539	0.1867
Stilbestrol-treated*	2	15%	0.1415	0.1739
Hypophysectomized †	2	18%	0.1083	0.1338
Hypophysectomized and given stilbestrol†	2	18%	0.1045	0.1313

* The stilbestrol was injected after twenty-four hours of fasting (5 mg. per hundred grams of body weight).

† These animals were put to death fifty-four hours after hypophysectomy.

Relative hypertrophy of the adrenals was observed in both the starved normal and the stilbestrol-treated animals. The single injection of stilbestrol resulted in no absolute enlargement of the adrenals. James and Nelson [38] and Ingle [44] have reported adrenal hypertrophy following repeated injections of stilbestrol. Loeser [39] observed enlargement of the glands and loss of lipoid following daily administration of 1 mg. of stilbestrol for two weeks. The glands of the stilbestrol-treated animals in the present small series manifested a moderate degree of depletion of lipoid of the zona fasciculata (the sudan appearing as fine droplets) in contrast with the untreated similarly starved controls (figs. 13 and 14). Numerous vacuolated non-lipoid-containing cells were seen in the outer portion of the zona fasciculata. Fry,[45] studying the metabolism of animals given injections of stilbestrol, observed evidence of increased

43. Korenchevsky, V., and Ross, M. A.: Brit. M. J. **1**:645, 1941.

44. Ingle, D. J.: Endocrinology **29**:838, 1941.

45. Fry, E. G.: To be published.

glyconeogenesis, as did James and Nelson [38] in experiments conducted over longer periods.

Hypophysectomy followed by a fifty-four hour fast resulted in considerable atrophy of the adrenal. No marked difference in reduction of lipoid content could be discerned between the adrenals of hypophysectomized, stilbestrol-treated rats and those of animals simply hypophysectomized (figs. 15 and 16). Fry found that whereas a single injection of stilbestrol into normal starved animals stimulated glyconeogenesis, there was no evidence of such stimulation in hypophysectomized starved animals. On this basis Fry suggested that the stilbestrol action on the adrenal is mediated through the pituitary. This idea is supported by the work of Bourne and Zuckerman,[12] who showed that the expected adrenal hypertrophy following injection of estrone (theelin) did not occur in hypophysectomized rats. They stated that "the effects of estrogenic stimulation on the adrenals are mediated through the anterior lobe of the pituitary." It is interesting to note the reduction of cortical lipoid following a single injection of stilbestrol in only those animals manifesting increased glyconeogenesis.

SUMMARY

An attempt has been made to correlate morphologic changes in the adrenal cortex of the rat with various experimentally produced metabolic and endocrine disturbances.

The administration of desoxycorticosterone acetate (2 mg. daily for thirty days) resulted in considerable atrophy of the adrenal in the male but no significant atrophy in the female adrenal. In both, the zona glomerulosa was shrunken and depleted of lipoid. Similar depression of the muscle and serum potassium by dietary measures resulted in no significant gross or microscopic changes in the adrenal cortex.

The adrenal atrophy observed following hypophysectomy was seen to be due to shrinkage and disappearance in part of the cells of the zona fasciculata, the zona glomerulosa becoming slightly hyperplastic—i. e., a histologic picture completely different from that seen following the administration of desoxycorticosterone acetate. The latter when given to hypophysectomized animals caused further atrophy of the adrenal, associated with shrinkage and depletion of the lipoid of the zona glomerulosa. These observations would indicate that adrenal atrophy following administration of desoxycorticosterone acetate is not mediated through the pituitary gland.

Castration of adult female rats caused no significant atrophy of the adrenal. Administration of desoxycorticosterone acetate to castrated females produced no further alteration in size but produced depletion of the lipoid of the zona glomerulosa.

Acute inanition resulted in moderate adrenal hypertrophy in the female and only slight hypertrophy in the male. Depletion of cortical lipoid was seen in about half the animals starved for one week. Hypophysectomized rats suffering from decreased appetite with resulting extreme loss of weight over a four week period exhibited adrenals smaller than those of comparatively well nourished hypophysectomized animals. This would indicate that factors other than lack of adrenotropic hormone may be responsible for the adrenal atrophy seen in chronic inanition.

The adrenals of rats fed a diet rich in protein were markedly enlarged and showed some degree of lipoid depletion. The latter was characteristic of the adrenals of rats receiving a single injection of stilbestrol. It is suggested that adrenal hypertrophy and lipoid depletion are indicative of the increased protein catabolism observed in acute inanition, with increased consumption of protein and after administration of stilbestrol.

PARVILOCULAR TUMORS OF THE OVARY

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The majority of the investigations of ovarian tumors which have been published during the last twenty years have been devoted to the so-called rare solid tumors—the granulosa cell tumor, the dysgerminoma and the arrhenoblastoma. The cystic tumors, which statistically form the majority of all ovarian neoplasms, have been left in the background. However, these tumors which by frequency and size aroused much interest among gynecologists and pathologists during the last two decades of the nineteenth century still offer many interesting and unsolved problems. Some special types of cystic tumors have been entirely neglected in the recent literature. Among these is a type of cystoma the classification of which as a specific entity goes back to Pfannenstiel,[1] who in 1907 described a special type of ovarian adenoma which he called a "solid" adenoma.

This "solid" adenoma, according to his definition, is an ovarian tumor which grossly looks solid but microscopically presents innumerable small adenomatous or tubular formations embedded in a fibromatous stroma. This type of structure can be found in several groups of ovarian tumors. Pfannenstiel was the first to call this tumor parvilocular cystoma. The first case of his new group he described as follows:

> . . . In pseudomucinous cystomas of larger size sometimes solid parts can be found, which microscopically consist of very small cystic cavities lined by one row of typical pseudomucinous epithelium and separated from each other by a small amount of connective tissue. The little cysts contain pure pseudomucin. Sometimes the "solid" part predominates over the cystic, so that the tumor correctly may be called a "solid" adenoma.

Pfannenstiel mentioned a patient with a tumor of this type operated on by Werth: A 38 year old quadripara presented on the right side a pedunculated ovarian tumor weighing 10 Kg. It had a smooth surface and consisted partially of small cysts and partially of "solid" portions. The "solid" parts, which he illustrated beautifully in color, presented the typical parvilocular structure: small cystic cavities lined with pseudomucinous columnar epithelium. The tumor was histologically

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This investigation was aided by a grant of the Committee on Scientific Research of the American Medical Association.

1. Pfannenstiel, J., in Veit, J: Handbuch der Gynäkologie, ed. 2, Wiesbaden, J. F. Bergmann, 1907, vol. 1, pt. 4.

and clinically noncancerous, and the patient was found to be in good condition fifteen months after the operation. Pfannenstiel mentioned 2 cases of this type in his own material. In the first the bilateral tumors had the size of a fist and there was abundant ascites; in the second case the left ovary had the size of a hen's egg, whereas the right was represented by a pseudomucinous cystoma the size of a man's head. Both patients were in perfect health ten years after operation.

Thus far, the parvilocular cystoma—a term which seems to be better than "microcystic cystoma" because it avoids repetition—seems to be well defined and characterized by the formation of a grossly solid parenchyma consisting of innumerable microscopic cystic cavities lined by a single row of mucin-producing epithelium and embedded in a scarce fibrous stroma. Pfannenstiel cited only 1 other case from the literature, the case reported in 1905 by Glockner.[2] That case, the classification of which has been viewed with much doubt by later investigators, will be analyzed later. Kermauner[3] in 1932 discussed the parvilocular cystoma. I have been unable to find any case reports or discussions of the parvilocular cystoma in the interval between Pfannenstiel's and Kermauner's presentations. Kermauner cited a case published in 1870 by Waldeyer,[4] but in that case carcinoma of the stomach developed simultaneously. The ovarian tumor, according to the description and the illustration, probably was a deposit of this carcinoma and not primary. Today pathologists would call it a Krukenberg tumor.

Kermauner pointed out that at the second gynecologic clinic of the University of Vienna in thirty years no patient wth parvilocular cystoma had been operated on. To fill this gap he described, offering four illustrations, a case which he called the Woyer case.[4a] After operation the specimen was submitted to me. The tumor had developed several years after the menopause and caused signs and symptoms of a mechanical type, being hard and resistant like wood and reaching the size of a man's head. The surface was smooth and slightly bosselated. On transverse cut the neoplasm showed a few little cystic cavities in a firm, solid fibroma-like stroma. Through a magnifying lens, innumerable pinpoint-sized cavities became visible, resembling closely the structure of dense pumice stone. Microscopic sections revealed a typical microcystic structure. The little round and oval cavities were lined with one row of epithelial cells varying in type from low columnar to cuboidal (fig. 1). The upper part of the protoplasm of this epithelium

2. Glockner, A.: Arch. f. Gynäk. **75**:49, 1905.

3. Kermauner, F., in Veit, J., and Stoeckel, W.: Handbuch der Gynäkologie, ed. 3, Munich, J. F. Bergmann, 1932, vol. 7, pt. 3.

4. Waldeyer, W.: Arch. f. Gynäk. **1**:252, 1870.

4a. He meant that the patient had been operated on by Dr. Woyer.

and the contents in the lumens of the cavities gave positive reactions when stained with mucicarmine. The patient, as far as I know, was in good health eight years after the operation.

In his résumé Kermauner said that thus far no case of cancerous parvilocular cystoma had been observed. The next and until today the last discussion of the parvilocular cystoma was given by Miller,[5] in 1937. In his classification of ovarian tumors he described the cases enumerated by his predecessors and some additional cases which probably belonged to other groups. Frankl's case 16, called by Frankl[6] himself a case of "fibroma ovarii adenocysticum pseudomucinosum" is not one of parvilocular cystoma, and his case 15, called by him a case of "fibroma ovarii adenocysticum carcinoides serosum, partim pseudomucinosum," is a typical case of Brenner tumor. Amann's[7] case

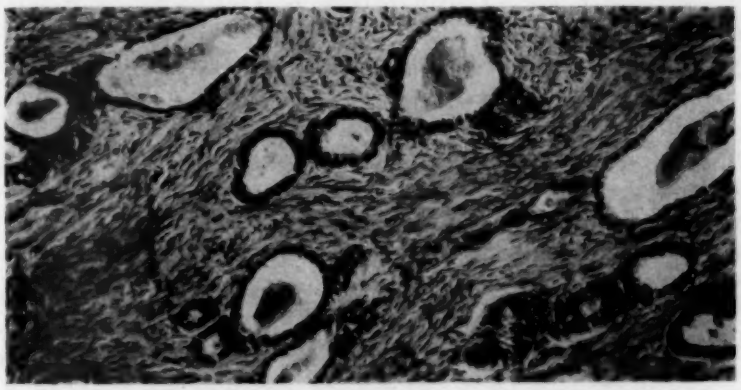

Fig. 1.—Dense fibromatous stroma with small cysts in the Woyer case. Some of the cysts are round; some are stretched by the surrounding fibroma-like bundles. The epithelium is low cuboidal with relatively large nuclei.

showed small cysts lined by mucin-producing high columnar epithelium, but this epithelium is shed and dissolved in its own secretion, which in some places penetrates the stroma and imbibes it. These two changes are typical for the pseudomyxoma or for Krukenberg's tumor but are not described in the parvilocular cystoma. Only the case reported by Orthmann,[8] as far as gross appearance of the tumor is concerned, may belong

5. Miller, J., in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1937, vol. 7, pt. 3.

6. Frankl, O.: Arch. f. Gynäk. **131**:325, 1927.

7. Amann, J. A., Jr.: Kurzgefasstes Lehrbuch der mikroskopisch-gynäkologischen Diagnostik, Wiesbaden, J. F. Bergmann, 1896; Monatschr. f. Geburtsh. u. Gynäk. **5**:224, 1897.

8. Orthmann, E. G.: Monatschr. f. Geburtsh. u. Gynäk. **9**:771, 1899.

to this group: a bilateral tumor, grossly solid, microscopically microcystic, pseudomucinous and clinically noncancerous. But here, too, the columnar epithelium dissolves in its secretion and many of the cystic cavities present no epithelial lining but have a plug of mucinous secretion embedded in the fibrillar stroma.

For the reason that probably different types of tumors are placed under the same heading, the statistics of some authors, as Lippert,[9] Kusuda [10] and Stratz,[11] are not reliable as far as the incidence of the parvilocular cystoma is concerned. "Solid" adenoma and "pseudosolid" cystoma evidently are confused, and solid adenoma, as well as the combination types of cystoma and fibroma, or cystofibroma or adenofibroma, admitted. This probably is the explanation why Stratz, for instance, finds that solid adenoma forms 3 per cent of all epithelial neoplasms of the ovary. If only those tumors are accepted as parvilocular cystoma which present the characteristics given by Pfannenstiel, this type of tumor proves to be rather rare, as has already been pointed out by Kermauner. In the cases reported by Pfannenstiel, Amann and others the follow-up observations to prove that the neoplasms were clinically noncancerous were carried on for only a few years. The illustrations are more or less diagrammatic, simplified drawings, most of which give no accurate picture of the cellular structure. Only the presentation of Kermauner is illustrated by three modern photomicrographs and a colored painting of the gross tumor; the follow-up is limited to six years, and neither the history nor the autopsy report are given.

REPORT OF CASES

CASE 1.—Dr. C. E. Galloway, Evanston, Ill., supplied the history and Dr. E. L. Benjamin, Evanston, Ill., the slides of this case. Mrs. H., 38 years old a Danish-born white woman, was admitted because of pain in the rectum. A left ovarian tumor was diagnosed and removed in September 1937, in the Evanston Hospital. Pain, with some loss of weight and fatigue, returned, and in December 1937 the remaining ovary and the uterus were removed. She did poorly, and pain developed along the right costal margin. On admission she was pale and thin. The heart and lungs were normal; the urine was normal; the Wassermann and Kahn tests were negative; the hemoglobin was about 60 per cent; the red blood cells numbered 3,400,000 and the white cells 8,000 per cubic millimeter. Cystoscopic and roentgen examinations gave negative results. The temperature was normal. She received 9,000 roentgens (r) to six abdominal portals and one transfusion, with some improvement, and was discharged in February 1938. In April she was readmitted, considerably emaciated, with marked abdominal tenderness and rigidity. The red blood cell count was 3,000,000; the white cell count, unchanged.

9. Lippert, W.: Arch. f. Gynäk. **74**:389, 1904.

10. Kusuda, S.: Arch. f. Gynäk. **124**:269, 1925.

11. Stratz, C. H.: Gynäcologische Anatomie: II. Die Geschwülste der Eierstöcke, Berlin, H. Kornfeld, 1894.

She received an additional 3,500 r to four upper abdominal portals but declined rapidly, with nausea, loss of weight and weakness, but without fever. She died in June 1938.

Specimen Obtained at the First Operation (left ovary and tube, examined by Dr. E. L. Benjamin).—The tube was normal with several serosal cysts at the tube and the mesosalpinx. The ovary measured 11 by 8.5 by 9.5 cm. It was covered by an intact, thickened, smooth, pearly gray to yellow-pink capsule. Numerous dilated and engorged vessels were seen within the capsule. The consistency was firm and the contour slightly bosselated. Cross section revealed a firm to semicystic, coarsely lobulated, yellowish green to pink-yellow, slippery, sticky tumor enclosed by a capsule and attached over an area of 8 by 9 cm. The internal lining

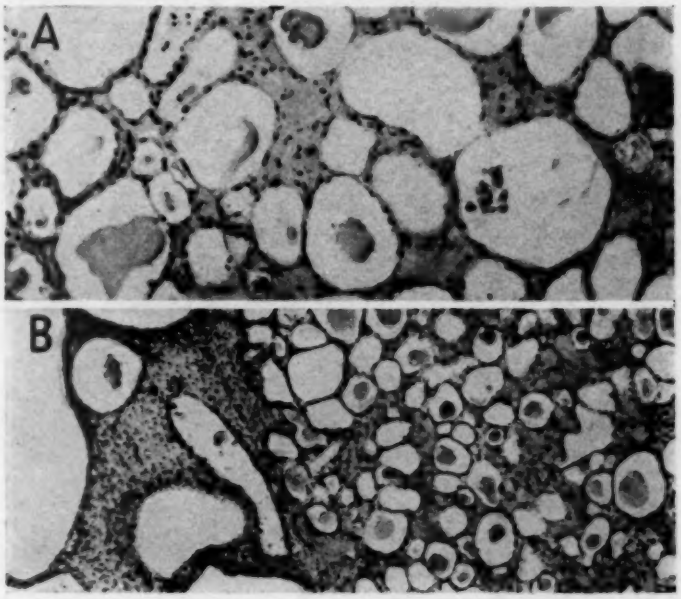

Fig. 2 (case 1).—Section of an ovarian tumor with small cysts as the outstanding structural feature; the stroma is poorly developed. The cysts are lined with low cuboidal or flattened epithelium. *A*, medium enlargement; *B*, low enlargement.

of the capsule varied from tissue paper thinness to a thickness of 2 mm., and it was incompletely studded by elevated translucent, glistening, white, discrete and confluent cysts.

The cut surface of the main tumor showed a firm, slippery and slightly sticky solid tumor through which passed yellow-gray streaks of fibrous tissue; other parts of the tumor were honeycombed and composed of numerous cysts filled with a sticky fluid. The walls were practically colorless. Still other solid areas of the tumor presented irregularly firm, pale, greenish yellow to pink-yellow areas. There was a moderate number of blood vessels coursing over the tumor, arising from its base and attached to the interior of the capsule.

Microscopically, the tumor presented different structures. Most characteristic were the parts which grossly looked solid. Here, corresponding exactly to the description and the illustration given by Pfannenstiel, were closely packed small round or oval cystic cavities, separated only by thin septums of fibrous stroma and lined by a single row of cuboidal or even flattened epithelial cells with vesicular nuclei and pale-staining protoplasm. These microcystic cavities presented two variations. In some areas they were larger and polyhedral. The single cavities had a diameter up to 8 mm. The larger ones were therefore visible to the naked eye. As the epithelium became higher and definitely cylindric, the upper part of the protoplasm was engorged by a pale secretion. In many cells the top was crowned by a crescent-shaped, markedly projecting drop of secretion. In some of the largest cavities proliferation of the epithelium resulted in a second and even a

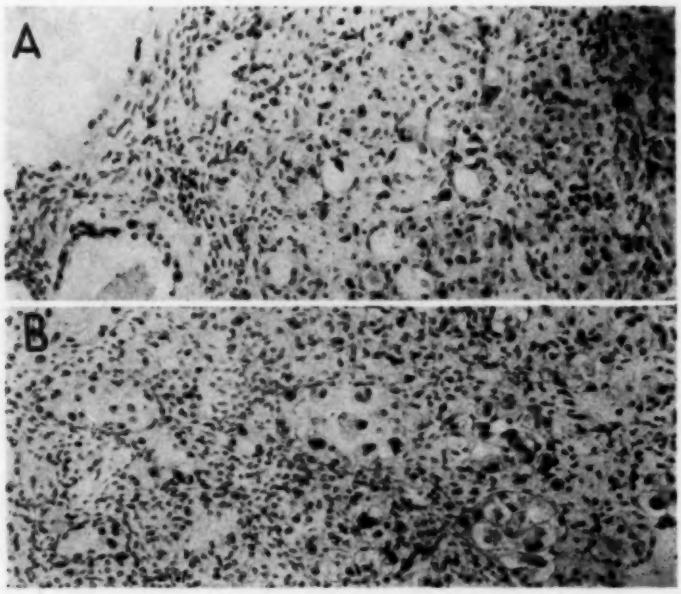

Fig. 3 (case 1).—*A*, transition from a cystic area into a solid medullary area; *B*, the solid medullary area. Note the irregularity of the nuclei, which are partly hyperchromatic.

third and a fourth row of cells. In other areas the cavities appeared smaller, the lumens being reduced in size by marked enlargement of the cells, which were of irregular shape and size and possessed pale protoplasm and hyperchromatic nuclei. In this way solid masses of epithelial cells were formed. These epithelial masses showed the carcinomatous character of the tumor in the irregular and atypical character of the individual cells and by invading and replacing the fibrous stroma. In some areas, where larger cavities were present, a third change could be noticed. The epithelial lining by piling up formed papillomatous projections which in the areas of greatest active proliferation filled out the cavities. These papillomatous projections also presented the cellular irregularity of a carcinomatous growth. As

a further proof of carcinomatous invasion, the same projections were found on the serosa of the uterus, on the perimetrium and on the surface of the second ovary. Here little clusters of dark-staining epithelial cells were embedded in masses of fibrin that covered the surface of the ovary. These represented the process of metastatic grafting to the surface, whereas in other areas there were papillomatous or small cystic deposits in the albuginea. In the small cysts the secretion in the lumen and in the upper parts of the cells gave a mucin reaction when stained with

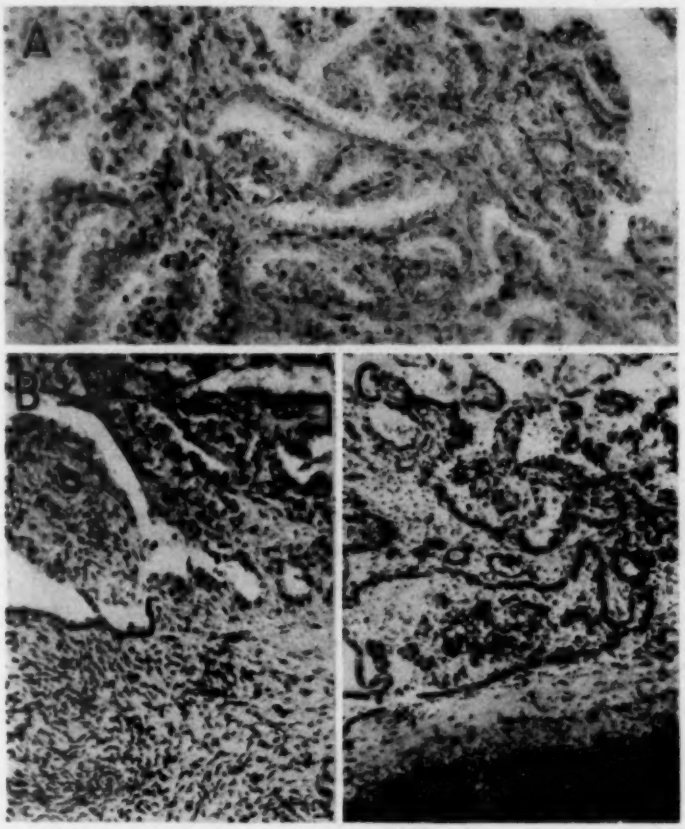

Fig. 4 (case 1).—*A*, area with adenopapillary structure; *B*, implantation of papillary deposits on the surface of the second ovary; *C*, implantation of papillary tumor masses on the thickened capsule of the spleen.

mucicarmine. Wherever the epithelial lining formed solid masses or papillary projections, the production of mucin gradually diminished and finally stopped (figs. 2, 3 and 4 *A* and *B*). Clinically, the patient gave full evidence of the carcinomatous character of this neoplasm, which was suspected after the first operation and became manifest in the deposits encountered at the second operation.

Autopsy (Dr. A. Brunschwig and Dr. N. B. Friedman, Albert Merritt Billings Hospital, Chicago).—All the abdominal viscera were matted and rendered adherent

to one another and to the parietes by fibrous and fibrinous adhesions and located between them were collections of fluid varying in color from greenish to dark brownish turbid and amounting to about 1,000 cc. The entire free peritoneal cavity was lost, the visceral and parietal surfaces being approximated and the cavities filled in by cystic tumor tissue and confluent masses bound together by dense fibrous and fibrinous adhesions. The intestinal loops were matted together, as were the pelvic viscera. The subdiaphragmatic spaces were similarly obliterated. The liver, gallbladder, spleen and pancreas were embedded in tumor tissue.

The abdominal as well as the periesophageal lymph nodes presented complete replacement of their parenchyma by tumor tissue. Microscopically, the tumor tissue consisted of proliferating papillary masses which morphologically duplicated the tumor tissue found at the perimetrium at the second operation (fig. 4*C*).

In its characteristic pseudosolid parts the tumor just described duplicates in every respect the description and definition given by Pfannenstiel. Small cystic cavities lined with mucin-producing low epithelium formed the specific structural unit. The change to larger cavities and papillomatous proliferation brings the tumor in relation to the common pseudomucinous cystoma, which forms larger, grossly visible cavities and eventually becomes papillomatous. This type ordinarily has higher and more regular columnar epithelium, which morphologically is identical with cervical epithelium. It may be that there is a relation between the height of the epithelium and the size of the cystic cavities such that high epithelium has both the tendency and the faculty to form large cavities, whereas low cuboidal epithelium tends to form small cavities. Whereas all the tumors of this type described by Pfannenstiel and Kermauner were noncancerous, this one pathologically, microscopically and clinically gave definite evidence of carcinomatous change. This is in accord with the general experience and conception that all types of ovarian tumors are primarily noncancerous but may change secondarily into cancer.

Case 2.—From Dr. Milton Bohrod I received six paraffin blocks of an ovarian tumor. Unfortunately, the history was lost, but in spite of this the histologic picture deserves comment (fig. 5). It represents a parvilocular cystoma with changes into papillary proliferation and into carcinoma as in the previous case. The only difference is that in some areas the single cells are markedly flattened, on both ends, so that the nucleus in the middle projects toward the lumen. The cells thus become hobnail shaped, the epithelium endothelium-like and the whole structure similar to that of a mesonephroma. However, the specific mesonephromatous glomerulus-like units are missing, and the mucin reaction is strongly positive, whereas that in the typical mesonephroma is negative.

Case 3.—Dr. L. Loeffler, pathologist of the Decatur and Macon County Hospital, Decatur, Ill., gave me the data on this case. A 69 year old white woman took sick four to five days before she died, with signs of intestinal obstruction.

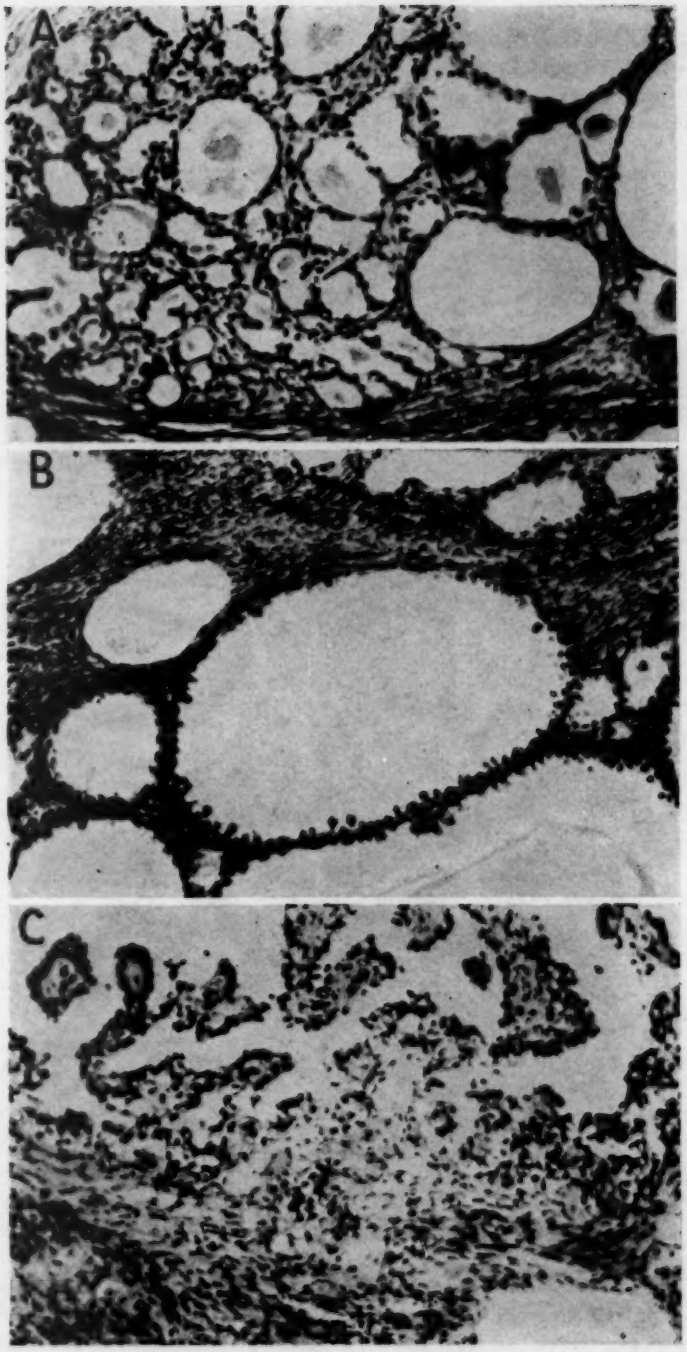

Fig. 5.—Sections of tumor in case 2: *A*, small cystic area; *B*, larger cysts, the stretched epithelium of which duplicates the hobnail or mushroom-shaped cells of mesonephroma; *C*, papillary area.

Roentgen examination showed a large soft tissue mass in the left side of the abdomen and the sigmoid narrowed where it crossed this mass. Removal of the tumor was not considered because of the bad condition of the patient. Two days after admission a small incision was made and a cystic part of the tumor emptied.

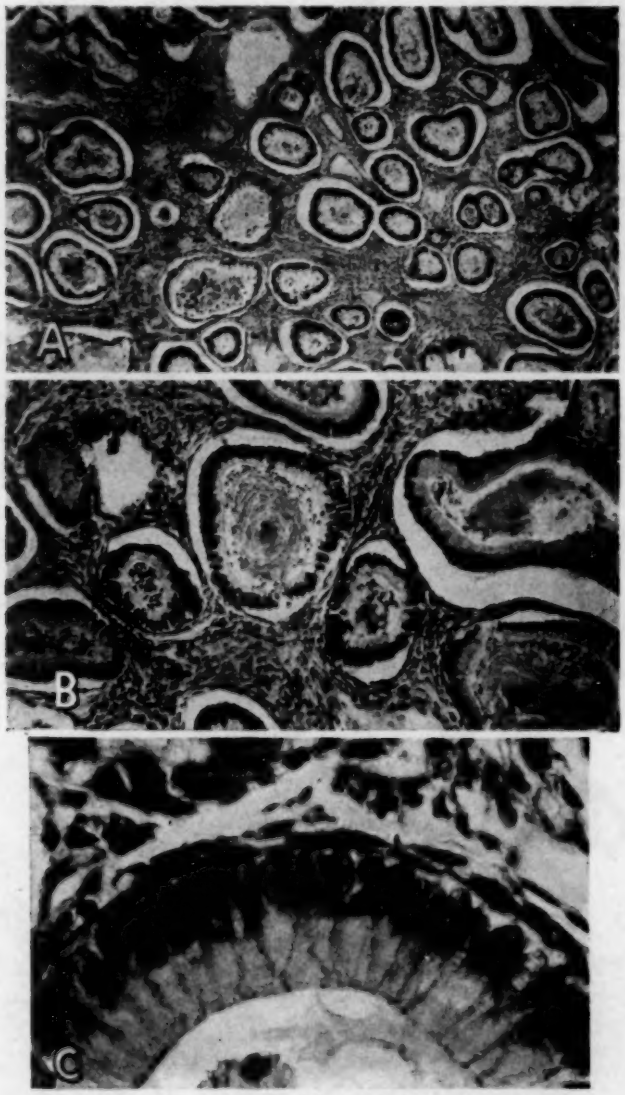

Fig. 6 (case 2).—Small round cysts lined with high columnar epithelium and embedded in well developed fibromatous stroma. (The separation of the epithelium from the stroma indicates that necrosis is beginning in consequence of the twisting of the pedicle.) *A* shows low enlargement, *B* medium enlargement and *C* high enlargement.

The patient died with symptoms of intestinal obstruction, heart failure and what was believed to be a twisted ovarian cystoma. The autopsy revealed a freely movable pelvic tumor about the size of a man's head, connected with veil-like fibrinous membranes to the surrounding tissues. The tumor was infarcted with mostly clotted dark red blood in its several chambers. The surface of the tumor was smooth, bluish and bloody and covered by a fibrous membrane. On the cut surface numerous smaller cysts were seen, about 4 by 5 cm. in diameter, all filled with blood. The solid masses were relatively soft and cut easily, and the cut surfaces were finely granular. The color of these masses was grayish pink. They were sharply defined within the limit of smaller cysts, about 3 by 4 and 2 by 2 cm. in size.

The tumor represented the right ovary and was twisted around its pedicle counterclockwise from the right over to the left abdominal side above the pelvis. The pedicle was made up of the broad ligament and the tube, which were deep red and hemorrhagic. The uterus was small and showed several small subserous fibroids about 1 cm. in diameter. The intestines were all pushed over to the right side of the abdomen, especially the sigmoid and the small intestines. The sigmoid crossed the abdominal cavity from the upper left to the lower right quadrant.

Microscopically, the tumor showed solid parts, which duplicated a moderately cellular fibroma, and cystic parts, which were composed of irregularly shaped cavities. The largest cavities were about 5 cm. in diameter. From these large cavities there were gradual transitions to the smallest, which measured 0.01 mm. in diameter. In large areas of the tumor the tissue was studded with small cysts, thus presenting the typical picture of the grossly solid, microscopically parvilocular cystoma (fig. 6). The lining of the cavities consisted of high columnar epithelium with oval nuclei filling the basal part of the cells. The protoplasm of the columnar cells, as well as the contents of the cavities, gave a strong mucin reaction. As a consequence of the twisting, extensive areas of the tumor were necrotic and congested with red blood corpuscles, and even in the well preserved areas the damage to the tissue was indicated by the separation of the epithelium from the stroma. There was no indication of carcinomatous change.

The tumor just described was a fibrocystoma with relatively small compartments, which by growing still smaller and being reduced to a size far beyond gross visibility formed the typical structure of a parvilocular cystoma. The fibrous stroma together with the large and small cavities forms the main part of the tumor tissue. These cavities are separated from one another by strong broad fibrous walls and not by paper-thin septums such as those seen in the multilocular serous or pseudomucinous cystoma. Occasionally a small area presenting the mucinous type of epithelium resembles closely the pseudomucinous cystoma. But in tumors of the latter type the columnar epithelium is higher and more regular and the protoplasm stains paler than in this case.

DIFFERENTIAL DIAGNOSIS

Pfannenstiel mentioned that pseudosolid structure can be found in ovarian tumors of different types, and Kermauner [8] in his book has an illustration (no. 88) of a pseudomucinous cystoma with a pseudosolid

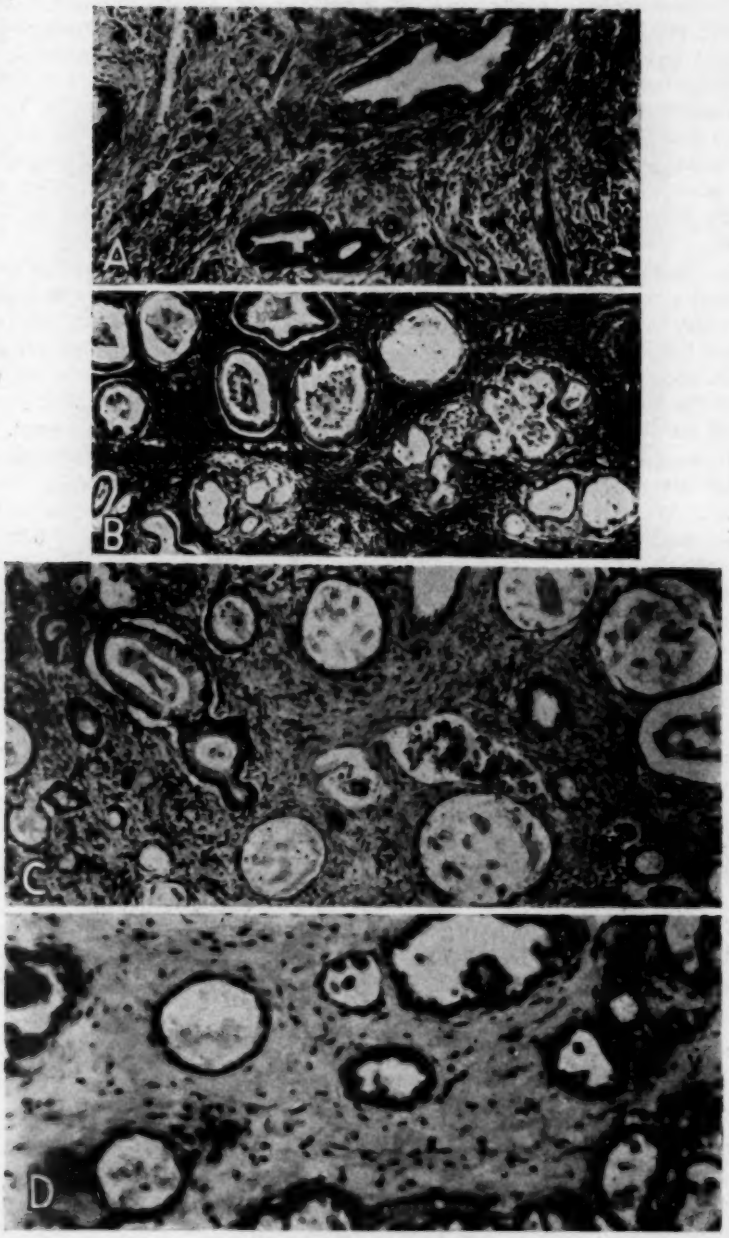

Figure 7

(See legend on opposite page)

node in the center which is not carcinomatous. Unfortunately, there is no histologic description in his text. Miller [5] described similar findings and cited cases described by Glockner,[2] Frankl [6] and Meyer.[12] A characteristic example of this type is in my collection:

A 61 year old woman showed the right side of the abdomen dilated by a smooth cystic ovarian tumor the size of a man's head, with solid immobile firm nodes in the pouch of Douglas. By laparotomy complete hysterectomy was performed, but the peritoneum of the vesicouterine fold and of the pouch of Douglas was found infiltrated and indurated by tumor tissue. On examination of the specimen the uterus was found to be of normal size and shape with polypoid hyperplasia of the endometrium, the tubes normal and the left ovary transformed into a multilocular pseudomucinous cystoma with a smooth surface. The right ovary was the size of a plum and showed on the transverse cut surface numerous, irregularly shaped small cystic cavities filled with mucin. On section the large tumor showed in its central basal part a fibrous thickening of the intercystic septums which had fused with remnants of ovarian tissue. The peripheral portion showed large cystic cavities lined with pseudomucinous, rather polymorphous columnar epithelium. This epithelium by its irregular and atypical elements gave evidence of carcinomatous change. In the basal area solid fibroma-like masses of stroma were found, which were studded with round cystic and elongated glandlike cavities lined with the same pseudomucinous carcinomatous epithelium. These cavities were seen in the periphery of some of the corpora candicantia and adjacent to the perivascular lymph spaces of some medium-sized arteries, as a result of carcinomatous invasion. The tumor in its central part grossly duplicated the parvilocular cystoma. Its histogenesis, however, was definitely different. It represented a combination tumor: a multilocular pseudomucinous cystoma plus a fibroma, with secondary carcinomatous change of the pseudomucinous epithelium, which finally invaded the fibromatous part of the tumor (fig. 7 *A* and *B*).

This tumor definitely belongs to a group of ovarian neoplasms different from the parvilocular cystoma. It is similar to the latter only in the gross appearance of some of its parts. Histologically and histogenetically it represents a different entity and has to be classified as pseudomucinous papillary fibroadenocystoma with secondary carcinom-

12. Meyer, R.: Monatschr. f. Geburtsh. u. Gynäk. **44**:302, 1916.

EXPLANATION OF FIGURE 7

A and *B*, carcinomatous fibrocystoma: *A* shows carcinomatous tubules close to the hilus and *B* small cysts at the hilus lined with columnar or cuboidal mucinous epithelium and embedded in fibrous stroma.

C, Krukenberg's tumor. The section is from a deposit of carcinoma in the ovary following metastasis of a carcinoma of the stomach to this site. Small cysts characterize it; the lining epithelium shows transitions from high columnar to flat, linear epithelium. A lymph space to the right from the center contains floating signet ring cells. The stroma is invaded by single carcinoma cells and small clusters.

D, carcinomatous mesonephroma of the ovary with single and confluent small cysts.

atous changes. Pseudosolid structures can be found in many other types of ovarian neoplasms which have to be separated from the parvilocular cystoma.

Multilocular Pseudomucinous Cystoma.—Here the cavities are much larger and may reach a diameter of several centimeters. But sometimes they may remain small and may form pseudosolid nodes of a spongy structure. Microscopically, the single cavities on these nodes are polyhedral, forming a honeycomb-like network, and the interstitial tissue is limited to a few connective tissue fibers in the thin septums between the cavities—the true parvilocular cystoma has a well developed fibroma-like stroma in which the round cavities are embedded. The epithelial lining of the pseudomucinous cystoma is high columnar; the epithelium of the parvilocular cystoma is much lower and may even be flat. The epithelium of the pseudomucinous cystoma eventually secretes distinct drops in goblet cells; in the parvilocular cystoma the mucinous secretion diffusely fills the upper part of the protoplasm.

When becoming papillomatous, the pseudomucinous cystoma in general forms large projections with a well developed stock of massive fibrous tissue. The parvilocular cystoma forms fine or thin ramifying projections which consist mainly of epithelium arranged around a thin core of a few connective tissue cells.

Krukenberg's Tumor.—This tumor sometimes presents areas composed of round little cysts scattered over the proliferated dense fibromatous stroma. Here the presence of primary carcinoma of the stomach, of the intestines or the gallbladder is of decided importance for the differential diagnosis. Histologically, the Krukenberg tumor shows diffuse infiltration of the stroma by carcinoma cells, which by secretion may develop into signet ring cells; it shows carcinoma cells floating in lymph spaces and eventually signet ring cells in the lining of the cavities, which may be shed and dissolved in the mucin secreted in the lumen; such changes are not found in the parvilocular cystoma (fig. 7 *C* and *D*).

Mesonephroma.—The mesonephroma may form a network of small cavities lined with low flat endothelial-shaped cells. The similarity of the mesonephroma to the parvilocular cystoma is great, and it is likely that some ovarian tumors diagnosed and described as mesonephroma are true parvilocular cystomas. However, such a misclassification can be avoided by diagnosing as mesonephroma only tumors which present the specific structures of this entity, none of which can be found in the parvilocular cystoma: (1) the glomerulus-like unit, consisting of a small cystic cavity which contains one capillary loop, which is covered with columnar epithelial cells whereas the cavity is lined with low endothelium-like cells; (2) solid areas consisting of proliferated endothelial cells, which appear stellate and are connected with each other by fine filiform

projections. These two specific structures are definitely free from mucin, whereas the epithelium of the parvilocular cystoma shows distinct and even marked production of mucin. However, limited production of a mucinous secretion can be found in the small cystic cavities of the mesonephroma [13] in some cases, but the true mesonephroma never shows a mucinous secretion comparable to that of the pseudomucinous cystoma and the parvilocular cystoma. Staining with mucicarmine or thionine should never be omitted for differential diagnosis of mesonephroma. In the mesonephroma the stroma never develops sufficiently to form fibroma-like areas such as those found in the parvilocular cystoma. Although the linings of the small cavities in both tumors may sometimes have a confusing similarity, the true epithelium, with distinct contact lines between neighboring cells, of the parvilocular cystoma (even if the protoplasm is lower than the projecting nucleus) can be distinguished from the endothelial cells of the mesonephroma, which touch each other only by their sharpened ends (fig. 7 *C* and *D*).

Fibrocystoma.—The fibrocystoma which is discussed by Pfannenstiel and by Miller in connection with the parvilocular cystoma contains no pseudosolid parts, but solid parts only, which represent the fibroma component of this mixed ovarian tumor. Here one finds not only thickening of the septums between the cystic cavities but well developed masses of solid connective tissue identical with the tissue of which the pure fibroma consists. The epithelial lining of the cavities is identical with the lining of the serous cystoma and consists of columnar cells with cilia or else of nonciliated columnar cells which secrete a serous fluid. This epithelium duplicates the tubal mucosa. Frequently the fibrocystoma produces papillomatous projections in the walls of the cysts and at the outer surface. These projections are plump and massive and have a heavy core of fibrous stroma, in contrast with the fine or thin ramifying papilli with scarce stroma found in the parvilocular cystoma. The fibrocystoma sometimes shows glandlike folds of the epithelium which extend into the stroma. These are never found in the parvilocular cystoma. The accurate name for the fibrocystoma for classification is "serous fibrocystoma," eventually with the attribute "papillary," "adenomatous" or "adenopapillary" (fig. 8).

Other Types of Ovarian Tumors for Differential Consideration.—For the histogenesis of the parvilocular cystoma experience and observations are still missing. The faculty of its epithelium to produce mucin brings it in close relation to the pseudomucinous cystoma, which is supposed to develop from inclusions of the ovarian surface epithelium with potency for prosoplastic changes in the direction of müllerian cervical mucosa. However, the production of mucin is not specific and

13. Schiller, W.: Am. J. Cancer **35**:1, 1939.

has been observed also in the epithelium of the rete ovarii. The possibility of papillomatous proliferation is one more property the pseudomucinous and the parvilocular cystoma have in common. For the time being it may be justified, further investigation pending, to place parvilocular cystoma in the classification of ovarian tumors as a subgroup of pseudomucinous cystoma.

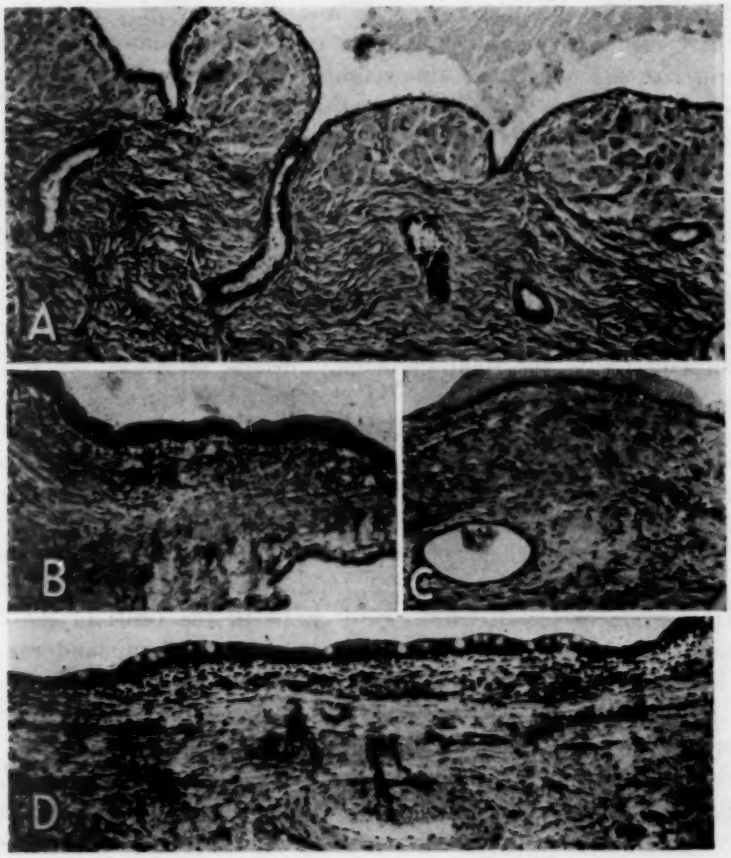

Fig. 8.—Fibrocystoma of the ovary: *A*, large cystic cavities in a well developed fibroma-like stroma. The folds when cut across simulate little cysts. Beneath the low cuboidal epithelium a small zone of pseudoxanthomatous cells has developed. *B*, high columnar, ciliated serous epithelium with dense protoplasm and elevated nuclei. *C*, high columnar mucinous epithelium with light protoplasm and short oval nuclei. *D*, serous columnar cells mixed with goblet cells.

As mentioned, Pfannenstiel [1] cited in addition to his observations only 1 other case, the case published by Glockner [2]:

This was a right-sided ovarian tumor, the size of a man's head, removed by laparotomy from a 47 year old tripara with normal adnexa uteri on the left side.

It measured 22 by 14 by 9 cm. and on section was perfectly solid. Sections revealed a fibromatous stroma with densely arranged, partially racemose glandular ducts lined by a low epithelium of probable mucinous character. These ducts presented a fanlike arrangement, being separated by septums which radiated from the capsule.

Whether this case is to be classified as one of parvilocular cystoma seems doubtful. Kermauner [3] did not accept this classification and suggested calling it an instance of adenofibroma developing from the rete. There is no question that the glandlike ducts of Glockner's illustration

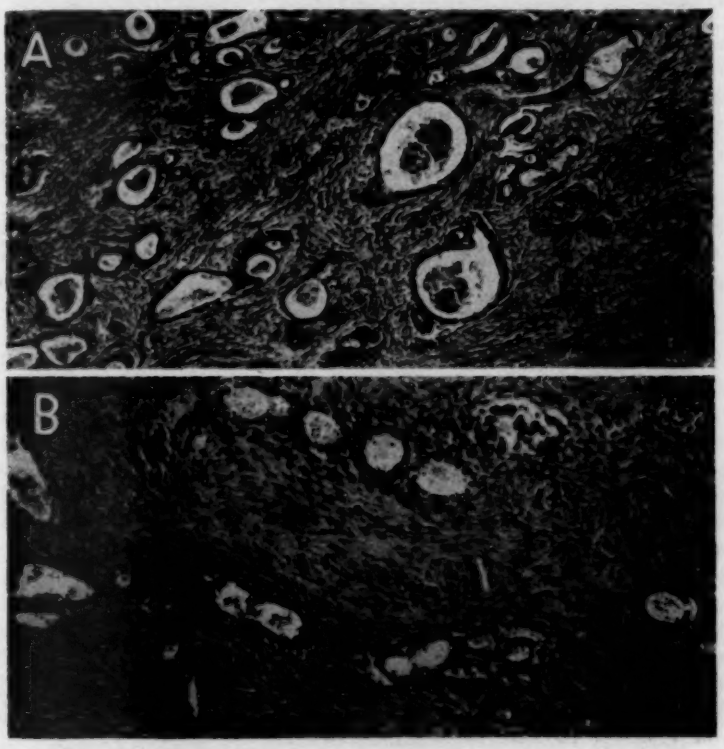

Fig. 9.—Ovarian tumor with fibromatous stroma and small round cysts lined with cuboidal epithelium: *A*, low enlargement; *B*, medium enlargement.

are different from the cystic cavities of the parvilocular cystoma, and, on the other hand, the parvilocular cystoma is not partitioned by radiating septums. Glockner's tumor probably represents a specific entity the characteristics of which are: small racemose-gland-like ducts lined with low columnar or cuboidal epithelium and embedded in a fibroma-like stroma. A specimen similar to Glockner's but without septums is in my histologic collection:

At autopsy on a 77 year old woman who died of lobular pneumonia, complicated by ulcerative esophagitis caused by monilia and atrophy of the right arm following

poliomyelitis, there were discovered incidentally a small serous cystoma of the right ovary and a stony-hard solid tumor, the size of a man's fist, which had replaced the left ovary. This tumor had a smooth surface and grossly gave the impression of a solid fibroma. Microscopically, the fibromatous stroma was densely studded with innumerable narrow, partially racemose ducts lined by cuboidal epithelium. There was little mucinous and considerable serous secretion in the lumens. Fat stains revealed sudanophil droplets in the protoplasm of some of the connective tissue cells in the stroma (fig. 9).

Another case of this group I owe to Dr. E. R. Pund and Dr. Robert Greenblatt, of the University of Georgia:

A Negro woman aged 43 years was admitted to a hospital because of acute heart failure with ascites. A pelvic tumor was found. There had been one child by her first husband and no pregnancies by her second. Her feet swelled on standing. The blood pressure was 200 systolic and 110 diastolic. Paracentesis was done, and 3,500 cc. of greenish fluid was withdrawn and injected into a guinea pig, with a negative result. Following the paracentesis, an indefinite movable mass, the size of a grapefruit, could be felt in the pelvis. Preoperatively there had been no menses for seven years, the menses having suddenly stopped at the age of 37. Three days' spotting was noted before operation. At laparotomy, bilateral firm smooth ovarian tumors, 14 cm. in diameter and well encapsulated, were found. One of these identical tumors contained a compressed simple cyst, 8 by 1 by 3 cm.

The patient was seen one year after operation and was then perfectly well.

Microscopically, the homogeneous fibroma-like stroma contained racemose strands consisting of two rows of epithelial cells, varying in type from cuboidal to columnar. Most of these strands were solid; some, however, formed a small lumen at the blind end. Some of the strands enclosed between the two epithelial rows single large round pale cells, hydropically swollen, with small nuclei like Call-Exner bodies. The strands or ducts were arranged in small groups or lobuli, being separated by more or less distinct septums of parallel fibers of connective tissue which resembled the septums in Glockner's case. Fat stains showed fine sudanophil granules in some of the cylindric cells of the trabeculae and in some of the spindle-shaped cells of the fibromatous stroma. The presence of fat granules in the cells of the strands constituted a similarity to what one finds in some cases of arrhenoblastoma. The fatty degeneration of the stroma cells was entirely different from the lipoid storage in the groups of large polyhedral Leydig cells present in the arrhenoblastoma with endocrine activity.

Here, for differential diagnosis, trabeculated granulosa cell tumor and trabeculated arrhenoblastoma have to be ruled out. In a trabeculated granulosa cell tumor, the cellular elements are round and never columnar, and the trabeculae are composed of more than just two rows of cells and have no tendency to form a lumen. In an arrhenoblastoma, the columnar cells which compose the trabeculae are much higher, the trabeculae are accompanied by Leydig cells, which here are missing, and form only short strands, never a large, well worked-out network as in this tumor. In almost every case of the trabecular granulosa cell tumor or of the trabecular arrhenoblastoma, areas of lower maturity similar to cellular fibroma can be seen with gradual transitions to

trabeculated parts of higher maturity. Such areas were not present in this tumor. Evidently this tumor represented an earlier phase of the glandlike ducts, during which phase they are still solid. This phase

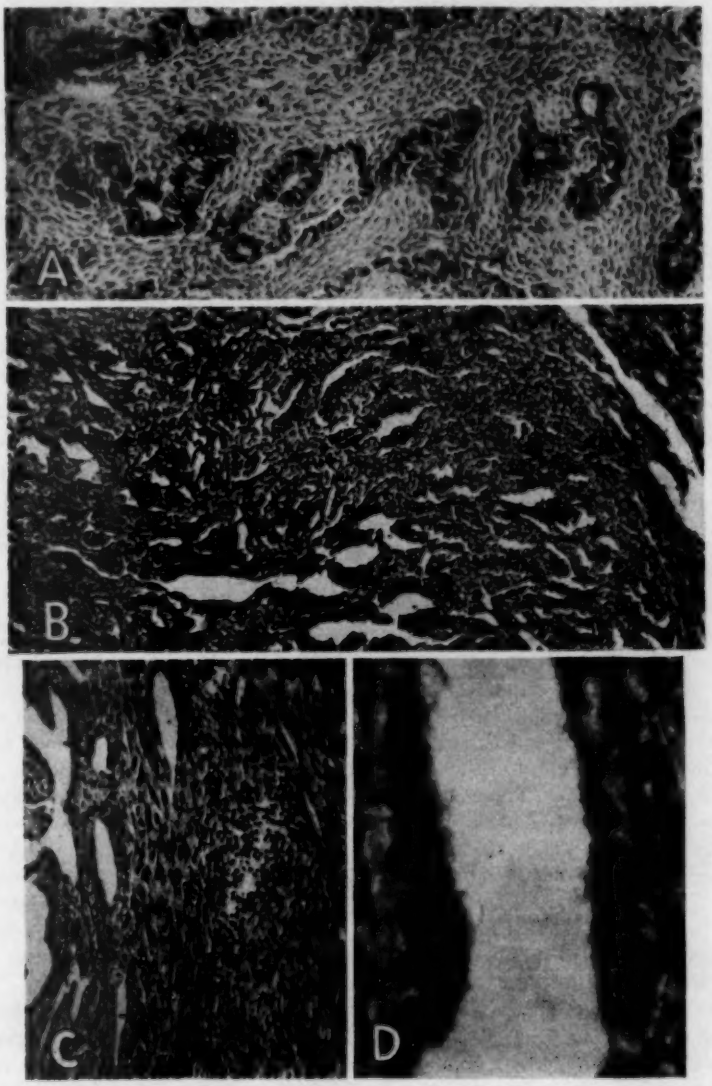

Fig. 10.—*A*, ovarian tumor with solid anastomosing ducts in fibromatous stroma and beginning formation of lumens in the thickened bud-shaped ends. The structure duplicates the fetal phase of the rete. *B*, *C* and *D*, ovarian fibroma with short, partially spindle-shaped ducts which anastomose at small angles. Note the distinct cuboidal epithelium. The shape of the ducts and the epithelium duplicate the rete ovarii.

is to be compared to the phase of solid strands which in embryonic development precedes the formation of a lumen in almost all the ducts. The relation of the two types corresponds to the relation between the trabeculated immature arrhenoblastoma with thin solid strands and the mature testicular adenoma with cordlike ducts in which distinct lumens have developed (fig. 10 *A*).

A case with a still younger earlier phase in the development of the epithelial constituents of the tumor has been described by me in a paper [14] which deals with the origin of the Brenner tumor from the rete ovarii: This case was one of multilocular serous fibrocystoma. The tumor was the size of a man's head and showed large cystic cavities containing serous fluid, lined with a high columnar, partially ciliated epithelium and separated by thick fibromatous septums, which in certain areas were as much as a thumb's width in thickness. Close to the hilus, corresponding to the location of the rete, the fibromatous stroma showed numerous islands of densely arranged cells. These cells had rather large dark nuclei and small amounts of protoplasm. They underwent transformation into epithelial elements. This phase is to be correlated with the formation of the rete from the local stroma: first by transformation of the local mesenchymal cells into solid medullary cords and later by transformation of these cords into the channels of the rete, as Fischel [15] has described.

Dr. Otto Saphir, of Chicago, supplied the history and the slides of a case in which the tumor presents a still greater similarity to the normal rete ovarii: A 59 year old white woman, eight years past the menopause, complained of pressure in the suprapubic region and constipation for three months. At laparotomy a large fibroma-like tumor replacing the left ovary was removed. The uterus was normal in size and contained an intramural fibroid, the size of a hazelnut. Tumor nodules were found on the serosa of the small intestine, and one in the omentum, the latter the size of an orange. The right ovary seemed to be normal in size and shape.

The specimen consisted of a massive tumor, weighing 920 Gm. and measuring 15 by 12 by 8 cm. The external surface was mottled yellow-gray, red and purple and was ragged. The tumor parenchyma was nodular and of a semifirm consistency. The sectioned surface was grayish pink with multiple hemorrhagic dots. Also present was a small similar tumor node, measuring 6 by 4.5 by 2 cm., of the same structure. Adherent to this were irregular pieces of fatty tissue (mesentery).

The microscopic sections of the ovarian tumor presented a cellular, well vascularized fibroma. In many areas the spindle-shaped cells were getting shorter and possessed a small amount of protoplasm only; the nuclei of these cells were largely hyperchromatic and of irregular, even bizarre shape. This transformation indicated the sarcomatous change which caused the intraperitoneal deposits. In the region of the hilus the fibromatous stroma included a network of cavities like channels or sinuses, which anastomosed at sharp angles and were lined with an indistinct

14. Schiller, W.: Arch. f. Gynäk. **157**:65, 1934.

15. Fischel, A.: Lehrbuch der Entwicklung des Menschen, Berlin, Julius Springer, 1929.

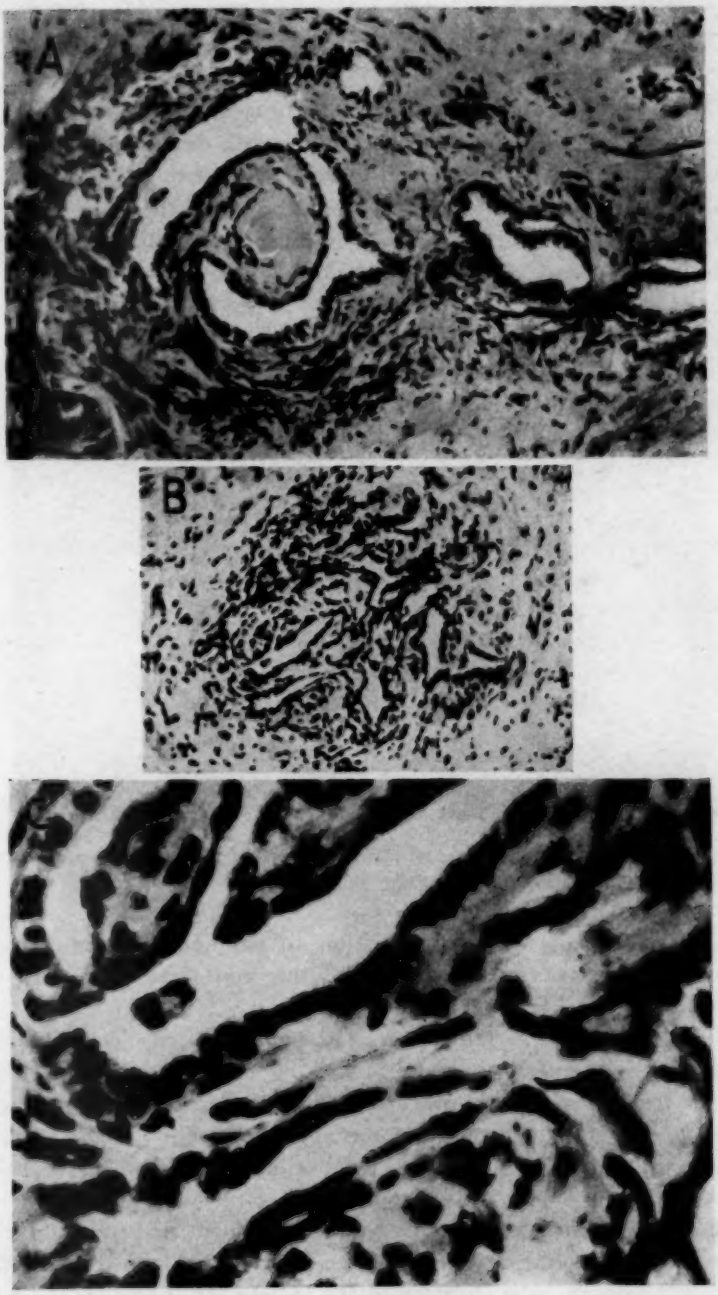

Fig. 11.—*A* and *B*, examples of postclimacteric rete; *C*, epithelium of *B* under high power.

single row of little cuboidal cells with pale protoplasm and lightly staining nuclei. This system of cavities gave the impression of a magnified proliferating rete ovarii (figs. 10 *B*, *C* and *D* and 11).

These 4 tumors evidently present an entity characterized by ductlike and glandlike inclusions embedded in a well developed fibroma-like stroma. They are different and have to be distinguished from the parvilocular cystoma. The differences are: first, there are no round or oval cavities but narrow strands or ducts; second, the fibromatous stroma forms a comparatively much greater part of the tumor parenchyma, and third, the mucinous secretion is not present in all cases and if present is not distinct. The ducts and glands have such small lumens that grossly, even when examined with a magnifying lens, the tumor seems to be solid and consequently in all instances has been diagnosed

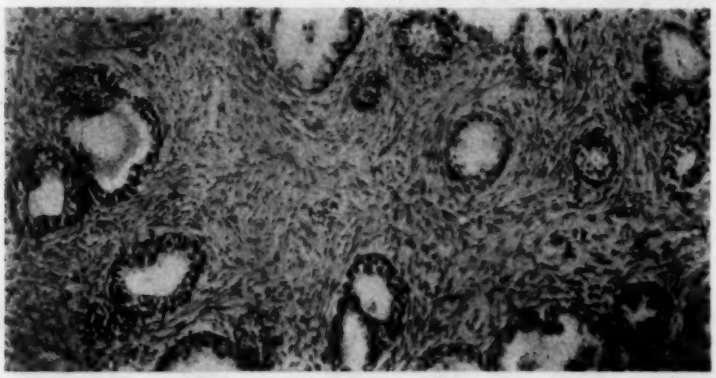

Fig. 12.—Hyperplastic prostate of man 76 years old. In appearance the stroma and cysts duplicate parvilocular ovarian tumors.

as simple fibroma. Neither transition to papillomatous proliferation nor carcinomatous transformation of the epithelial part was ever observed.

The similarity of certain areas in some of the parvilocular ovarian tumors to a hyperplastic prostate is striking (fig. 12). No conclusion, so far as the histogenesis is concerned, can be drawn for the time being. The area where the prostate and the area where the gonads form are too far from each other for one to consider fetal misplacement, and there is no evidence to support a teratoid origin of the tumors.

The parvilocular adenofibroma contains no hormone-producing tissue and consequently produces no endocrine interference with the menstrual cycle. It produces no virilization of the patient. For this reason it has to be separated from the testicular adenoma, although a certain morphologic similarity cannot be overlooked, a similarity which is due

to the similarity between fetal rete and fetal germinal cords. However, in the testicular adenoma, which represents the highest degree of maturity of the arrhenoblastoma, the interstitial tissue forms thin septums only and never fibromatous masses as in the adenofibroma. The arrhenoblastoma, particularly the trabeculated type, shows adjacent to the cords groups of Leydig cells, which are missing in the adenofibroma. The localization of the ducts and glands close to the hilus, as well as the morphologic similarity, makes it probable that the tumor develops from the fetal remnants of the rete. Thus, in the general classification of ovarian neoplasms the tumor has to be placed together with the granulosa cell tumor and the fibroma in the group of ovariogenic tumors. As a name I should like to suggest "adenofibroma" in the sense that a combination of fibroma and cystoma is called fibrocystoma or cystofibroma. To indicate the narrowness of the glands the attribute "parvilocular" may be added.

SUMMARY

The parvilocular cystoma is an ovarian tumor characterized microscopically by small cystic cavities lined by a mucin-producing epithelium and embedded in a fibrous stroma. Papillomatous proliferation and carcinomatous transformation may be observed. The parvilocular adenofibroma presents ducts and narrow glands embedded in a well developed fibroma-like stroma; it probably originates from fetal remnants of the rete ovarii.

FILARIAL EPIDIDYMOFUNICULITIS

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The medical departments of the Army and the Navy are emphasizing instruction in tropical medicine. The traditional concept that certain diseases are tropical, delimited geographically, is rapidly vanishing. Many diseases which are now widely disseminated originally had a restricted distribution.

World War II and modern facilities for swift travel have abolished the physical barriers that formerly isolated geographic regions, some of which have been from time immemorial the only foci of certain diseases. When American Expeditionary Force II returns home, many will have diseases unfamiliar to American physicians. New diseases will probably be introduced and spread, which the physicians of the United States must be prepared to recognize and take speedy measures to prevent.

Next to malaria, filariasis (Wuchereria bancrofti) is the most prevalent "tropical" disease. It is indigenous in almost all warm regions of the world, and it is extremely common in this nation's most important theaters of war in the Far East. In many of the islands of the Pacific 60 to 80 per cent of the inhabitants are infected. Like the malarial parasite, the filaria is introduced into its human host through a mosquito bite.

Filarial disease of the male genitalia is a distinct clinical condition and the most common manifestation of the infection in man. Patients with chronic swellings about the spermatic cord, testis, scrotum and groin who have been in the tropics should always be regarded as possibly filarial.

In the following cases biopsy revealed unsuspected filarial disease of the spermatic cord and epididymis.

REPORT OF CASES

CASE 1.—A foreign Negro seaman, aged 37, a resident of St. Vincent, Cape Verde Islands, was hospitalized because of pain in the back and in the scrotum. One month previously he had injured his back aboard ship. The pain in the scrotum was of about one month's duration. The general examination showed nothing of importance. The scrotum disclosed a hard nodular lesion of the right epididymis. One examiner interpreted this to be cysts of the epididymis, some of

which were calcified. The patient was aware of the lesion and stated that it had been present for ten months. He had an evening temperature of 37.2 to 37.4 C. (98.9 to 99.3 F.). The Kolmer and Kahn tests were strongly positive.

Exploration of the right scrotal sac revealed that the spermatic cord was thickened, matted and beaded with roughly ovoid stony-hard grayish nodules, 3 mm. to 2 cm. in diameter. Here and there little pouches filled with fluid were present. The epididymis was large and stony hard and presented a coarse hobnail appearance. Small hard nodules and vascularized hard plaques were present over the surface of the testis. The nodules cut like cartilage, and many

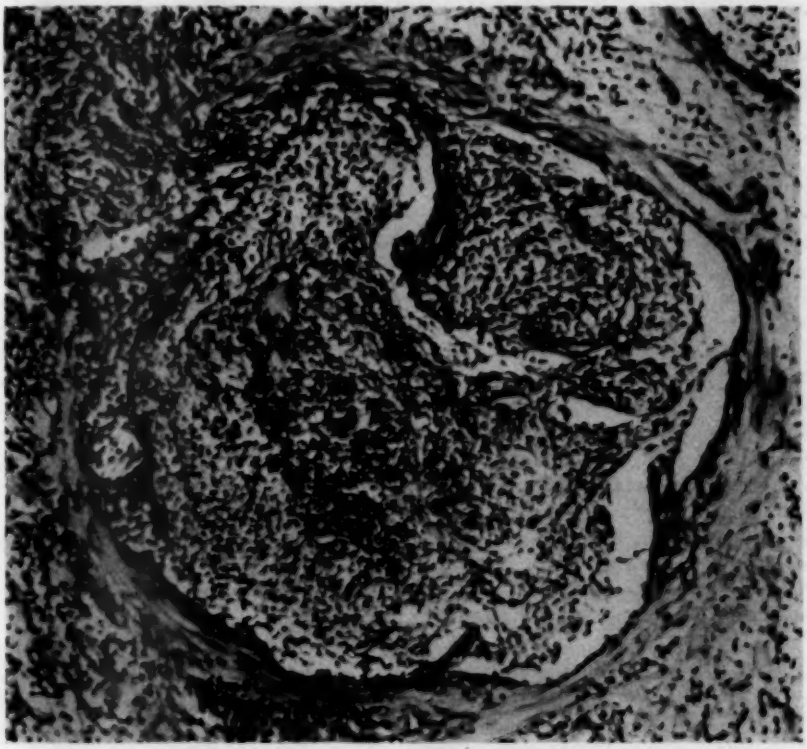

Fig. 1.—Granulomatous obliterative endolymphangitis with beginning focal necrosis.

were largely calcified. The saccules contained turbid yellowish watery fluid. Their interior presented a strawberry-like surface.

The condition was interpreted as a neurofibromatous lesion, and the cord and testicle were removed.

Histologic examination of various levels of the spermatic cord showed marked old interstitial fibrosis, fibrotically thickened capillaries and sparse infiltrations of plasma cells and lymphocytes. The adventitia of the vas deferens was moderately thickened but not otherwise altered. The arteries showed no significant changes. The veins showed muscular hypertrophy. Here and there small sausage-shaped, ovoid and piriform calcified bodies thickly encapsulated in hyalinized connective

tissue were present. Discernible lymphatic vessels showed sclerosis and more or less old obliterative lymphangitis. Some contained refractile crystalline plaques and calcified debris. The tumor nodules present in the gross specimen were formed of whorls and interlacing strands of hyalinized fibrous tissue. They contained calcified segments of filaria. Fibrotically thickened capillaries and focal infiltrations of plasma cells were present. The epididymis was entirely replaced by fibromatous nodules having the same structure. The tunica albuginea showed focal plaques of sclerosis sparsely infiltrated by plasma cells. The parenchyma of the testis showed no pathologic changes.

Sections of the saccules showed these to be fibrosing lymphoceles lined by fibrosing granulomatous tissue.

The diagnosis was chronic and obsolete filarial epididymofuniculitis.

The patient made an uneventful recovery.

Case 2.—A white foreign seaman, aged 28, a native of Fort de France, Martinique, was hospitalized Oct. 31, 1941 because of a swelling in the scrotum. Two and a half years previously he had an operation for hydrocele. He was well until fourteen days prior to admission, when swelling and tenderness developed in the left side of the scrotum. Examination disclosed nothing except for the changes in the scrotum and the left inguinal region. An old operative scar and a small hard swelling were present in the left groin. Both testicles were larger than normal and firm. The left epididymis was large and tender, and its head felt like multiple small cysts. A spermatocele was suspected.

The left scrotal sac was explored. The spermatic cord was transformed into a thick, deformed, tortuous, varicose trunk irregularly adherent to the surrounding structures. It merged inseparably with the epididymis, the head of which looked like a cauliflower. The remainder presented a quilted appearance. The testis appeared normal. The spermatic cord and the epididymis were so completely involved in the inflammatory process and the blood supply was so compromised that orchidectomy and high vasectomy were done. Several days after the operation the left femoral and inguinal lymph nodes became large and tender. Two femoral nodes, 1 by 2 cm. in diameter, were removed for histologic examination. Two days later severe lymphangitis developed over the anterior surface of the thigh from the groin to the knee.

Roentgen rays of high voltage were directed to the groin and the thigh. The lymphangitis subsided, but the lymphadenitis remained unchanged, and the nodes involved were tender. The operative incisions healed uneventfully. The red and white blood cell counts and the differential blood cell picture were within normal limits. The Kolmer and Kahn tests were negative. Plasmodium vivax was found in thick blood smears. Repeated examinations of fresh and stained, thin and thick films of blood obtained between the hours of 10 p. m. and 2 a. m. and at other times failed to show microfilariae.

Histologic sections disclosed lesions of the lymphatic vessels in various stages of development in the spermatic cord and the epididymis. Lymphangiectasia was prominent, and the large vessels showed moderate to marked hypertrophy of their wall. Occasional lymphatics showed endothelial hyperplasia and swelling. The majority showed various degrees of epitheloid cell granulomatous endolymphangitis involving short stretches or the entire intima. In some vessels the granulomatous reaction was villiform. Occasionally the lumen was subdivided into compartments separated by delicate granulomatous septums. Many vessels showed partial granulomatous obliteration. Some showed massive obliteration with recanalization, and others showed complete granulomatous occlusion. One or more Lang-

hans giant cells were frequently seen in the granulomatous lesions, and often these were densely infiltrated by plasma cells and eosinophils. Occasionally miliary eosinophilic abscesses were present. In some lymphatic vessels the granulomatous proliferation showed foci of fibrinoid degeneration. Others showed foci of sup-

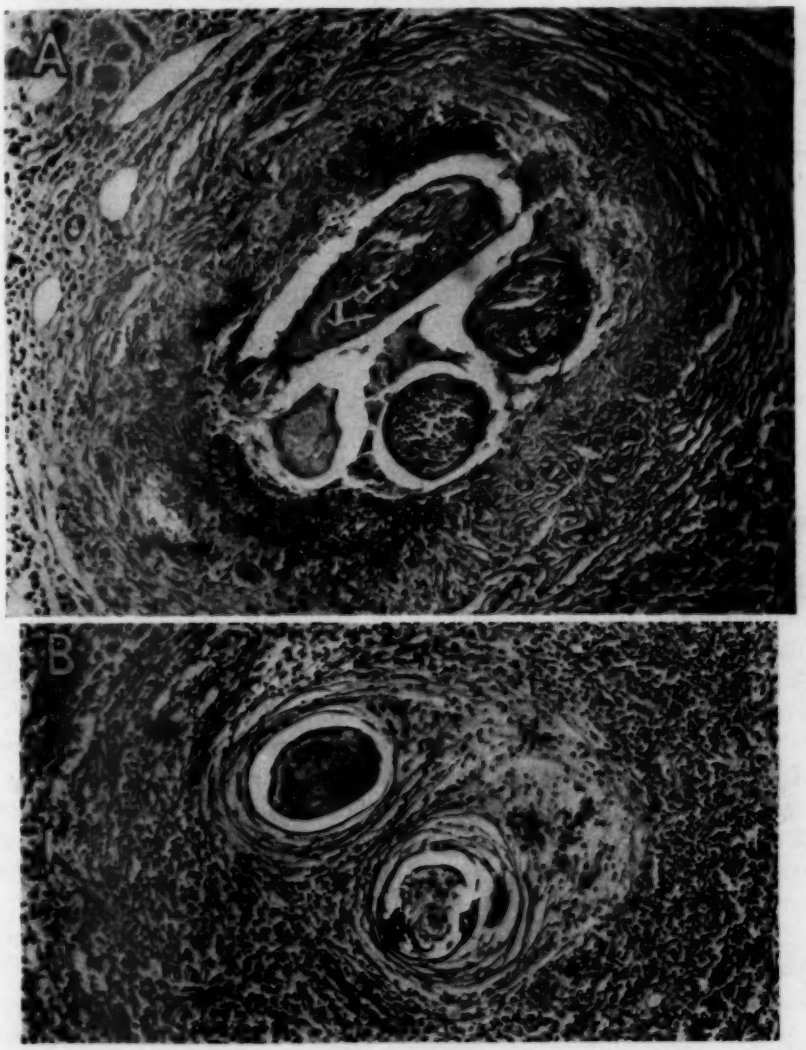

Fig. 2.—*A*, marked hypertrophic and fibrosing lymphangitis with gravid adult filariae. *B*, sclerosing lymphangitis with dead and calcifying worms.

purative thrombolymphangitis with more or less extension of the inflammation into the perilymphatic tissues. Bacteria were not demonstrable in these lesions. Some vessels showed more or less fibroplasia of their wall with lymphocyte, plasma cell and eosinophilic cell infiltration. Others showed advanced fibrohyaline thickening

with varying degrees of stenosis of the lumen. Here and there in dilated, granulating or fibrosed lymphatic vessels sections of gravid female filariae and remains of dead worms in various stages of disintegration and calcification were present. The cord, the epididymis and the adjoining tunica albuginea showed various degrees of active interstitial fibrosis, irregular edema, capillary vascularization with capillary endothelial swelling and fibrosis, areas of dense eosinophilic cell, plasma cell and lymphocyte infiltration, and islets of liposis.

The arteries showed no lesions. Some veins showed hypertrophy of their wall and minor to moderate degrees of adventitial fibrosis. The vas deferens showed no pathologic alteration. The tubules of the epididymis were dilated, and many contained spermatozoa. The testis showed some subcapsular and slight irregular interstitial lymphocyte and plasma cell infiltration.

The femoral lymph nodes showed old and active capsular fibroplasia, edema, capillary vascularization, stretches of dense lymphocyte and plasma cell infiltration, lymphangiectasia and obliterative granulomatous and fibrosing lymphangitis. The follicles were large and hyperplastic. Irregular hyperplasia of the endothelium of the sinus and obliterative granulomatous sinusitis were present. In areas there were conglomerate epithelioid granulomatous nodules with two to five Langhans giant cells. There were moderate diffuse capillary vascularization and fibrosis of the trabeculae and stroma.

The diagnosis was subchronic and subacute suppurative filarial epididymofuniculitis with granulomatous lymphadenitis.

COMMENT

In the male, filariasis manifests itself most frequently in the genitalia. The basic lesion is a characteristic obliterative granulomatous endolymphangitis. As a result of the lymphatic blockage, the microfilariae cannot enter the circulation and the parent worms die in situ. Some disintegrate and are absorbed; others calcify and are encapsulated, with the formation of hard fibromatous nodules. Frequently the earliest or the only evidence of the infection is a nodular or a cystic condition of the cord or the epididymis. Usually the patient is entirely asymptomatic. He may complain of the nodules in the scrotum or of the thickened cord, or the lesions may be detected during a routine physical examination. Involvement is frequently bilateral, with the lesions usually more advanced on one side.

In acute and subacute stages the granulomatous endolymphangitis is frequently associated with more or less suppurative inflammation. It is extremely doubtful whether bacteria play any part in the reaction.

The acute symptoms vary in severity, but pain, fever, chills and sweating are usually present. The patient usually has pain in the groin, the spermatic cord is swollen and indurated, the epididymis is swollen and very tender, and not infrequently an acute hydrocele develops. The scrotal skin may become swollen and edematous and the lymph nodes in the groin and the perilymphatic tissues acutely inflamed. The acute reaction may subside in a few hours or last

several days. The cord and the epididymis may remain indurated for several weeks. The hydrocele may be absorbed or it may persist.

The disease may become subacute, chronic and recurrent, or obsolete, with nodular or varicose changes in the spermatic cord or the epididymis as the only evidence of its occurrence.

In early stages, there is constant high eosinophilia, and microfilariae are demonstrable in the peripheral blood in a high percentage of patients. In searching for them the periodic and the nonperiodic species must be remembered. In the chronic and recurrent disease lymphatic blockage greatly reduces the chances of microfilariae reaching the blood stream, and in not more than 24 per cent of the cases of the disease in this stage are the findings positive.[1] Roentgenograms are useful in demonstrating calcified worms in the tissues. The parent worm and microfilariae are frequently present in the varicose lymphatics or in close proximity to them. Aspirated fluid may reveal the worms or chyle. In about 10 per cent of cases the hydrocele fluid contains microfilariae. Biopsy usually reveals the filarial nature of the lesion.

SUMMARY

With American Expeditionary Force II fighting in all parts of the world, and particularly with divisions in the Far Eastern theaters, where filarial infection is extremely common, attention is invited to filarial epididymofuniculitis, a frequent manifestation of filariasis. Two cases are reported.

1. Makar, N.: J. Egyptian M. A. **21**:682, 1938.

Case Reports

DISSEMINATED OSSIFICATION OF THE LUNGS

H. Gideon Wells, M.D., and Charles E. Dunlap, M.D., Chicago

Ossification of bronchial cartilages and of scars from tuberculosis or other lesions in the lungs is a common process. But diffuse forms of ossification, either ramified areas or diffusely scattered nodules, are rare. Manzini [1] reviewed the literature to 1938 and found but 43 cases, which he tabulated, and since that time but 2 more cases, confirmed by necropsy, have been found recorded. Strangely enough, not a single case report can be found in the English language, most of them being in German with a very few in French and Italian. Therefore it seems desirable to put on record a typical case and to discuss it from the standpoint of pathology briefly.

The diffuse form of pulmonary ossification occurs in two forms, racemose or branching (called *veraestelte* by the Germans) and nodular circumscribed (called *tuberöse* by the Germans). A third "diffuse" form, described by Cohn and included in most German classifications, is not diffuse but localized, and probably not related to the other forms. The racemose form is by far the commonest and consists of branching spicules of true bone running in the septums of the lungs, often continuous for some distance but with isolated spicules. Usually the process is limited to certain parts of the lung and is not truly diffuse. About 35 cases of this type have been described, which occurs almost exclusively in old men, in many of the cases affecting men of quite advanced years. The best explanation for this form is that advanced by Daust,[2] who stated that it appeared to be merely a metaplasia due to senile alterations in perivascular connective tissue. First the vascular media degenerates; then the perivascular connective tissue swells greatly and becomes hyaline and glassy. Following this, nuclei appear in the hyaline tissue, which are short and compressed, with dendritic processes, and apparently form osteoid tissue which becomes calcified with formation of bone. He speaks of preliminary calcification leading to bone formation, but nowhere in his or other articles is there found a description of calcification as a precursor of ossification. Rather a hyaline matrix of osteoid tissue is converted into true bone by the deposition of calcium. Marrow formation is occasionally observed in this new bone, but apparently it is much more rare and sparse than that commonly found in bone resulting from calcification of tuberculous scars or ossified bronchial cartilage.

The other form, the nodular circumscribed, or *tuberöse,* is distinctly different, both anatomically and in the class of patient involved, for it

From the Department of Pathology of the University of Chicago.

1. Manzini, C.: Riv. di pat. e. clin. d. tuberc. **12**:145, 1938.

2. Daust, W.: Frankfurt. Ztschr. f. Path. **37**:313, 1929.

3. Janker, R.: Fortschr. a. d. Geb. d. Röntgenstrahlen **53**:260, 1936.

affects chiefly relatively young persons with mitral stenosis. Janker [3] reviews the 7 cases reported to 1936 in which the condition was observed at necropsy and 3 cases in which it was observed only roentgenologically, and finds that 6 patients were aged from 20 to 30, 2 from 30 to 40 and but 1 between 40 and 50, this being in marked contrast with the average age of 67 in 23 cases of the racemose form collected by Daust. Furthermore, 7 of the patients had advanced cardiac disease. Anatomically, the lesions were characterized by being small, commonly less than 0.5 cm. in diameter, discrete and scattered widely through the lungs but especially near the pleura. Since this condition is observed almost exclusively in association with mitral stenosis, one would expect it to be associated with calcium-iron incrustations, but these seem not to be a precursor of the ossification, except perhaps in the case reported by Munk,[4] in which calcified elastic tissue elements heavily impregnated with iron were found as well as the ossified plaques. This form should be recognized roentgenologically, and Gross [5] reported that after his attention was called to the condition he observed 4 cases roentgenologically but did not confirm his observations by necropsy. One striking feature was the persistence of the lesion, for in 1 case it was observed to change but little in the course of eight years.

Nodular circumscribed ossification seems to be the result of connective tissue proliferation, both interstitially and within the alveoli, and would suggest interstitial pneumonia as the forerunner were it not for the fact that in most of the cases it occurs in lungs the seat of chronic passive congestion from mitral stenosis. Histologically, the lungs show marked thickening of the alveolar walls, not only with connective and elastic tissue increase, but sometimes conspicuously an increase of nonstriated muscle fibers. There is also usually observed within the alveoli an exudate of homogeneous fibrinoid material in various stages of organization. The bone occurs as lamellas, with thin small plaques, or as larger irregular masses involving both the interstitial tissue and the intra-alveolar connective tissue. No calcification is observed to precede the ossification, but rather osteoid tissue is first formed in which calcium salts are deposited. It is to be noted that in metastatic calcification of the lungs the calcium deposits do not undergo ossification, at least as far as we have seen or can learn.

The case that we have observed comes under the heading of diffuse circumscribed, or *tuberöse,* ossification.

REPORT OF A CASE

A woman aged 34 had suffered from mitral stenosis since childhood, with serious decompensation and marked edema on several occasions, and had been strongly advised against pregnancy. Nevertheless she became pregnant and appeared at the hospital at the seventh month in a somewhat decompensated condition. Her urine showed a trace of albumin, but there was no evidence of toxemia. She was given rest in bed and digitalis, under which she improved and went home to remain in bed until time for a cesarean section. On the day that she returned to the hospital there developed what was diagnosed as a thrombosis of the

4. Munk, E.: J. de radiol. et d'électrol. **23**:58, 1939.
5. Gross, A.: Fortschr. a. d. Geb. d. Röntgenstrahlen **58**:33, 1938.

iliac artery. Her child was delivered by cesarean section a few days before term. The operation was uneventful, but postoperatively her chest filled with rales and her temperature rose above the highest point on a clinical thermometer. She vomited terminally and died on the day after operation. Unfortunately, no roentgenogram of the chest were made.

At necropsy there was found a high grade mitral stenosis, the orifice barely admitting the tip of the little finger. There was a solitary red vegetation, 1 mm. in diameter, near the valve margin. Besides the findings of a recent cesarean section, a recent small mural thrombus in the abdominal aorta and acute passive congestion of the liver, the only abnormality was in the lungs. These showed acute bronchopneumonia and were dark purple. Lying in the visceral pleura over all lobes were minute flat plaques of ossification, averaging 1 mm. in diameter. Occasional calcific deposits similar to those in the pleura were present throughout all the lobes. No fibrosis was apparent about these areas of ossification. The pulmonary vessels were thick walled but not calcified, and there was no calcification in the peribronchial nodes. No brown pigmentation was discernible; if present, it was obscured by the congestion. No other organ showed any calcification whatever. On examination of roentgenograms made of the lungs after the necropsy there were found at least 200 opaque flecks, widely distributed through both lungs but more in the lower lobes than elsewhere. None of the flecks was over 4 mm. in diameter (fig. 1).

Microscopically, the lungs presented diffuse acute hemorrhagic bronchopneumonia, with minimum deposition of hemosiderin pigment, superimposed on an older process. The alveolar walls were generally but not uniformly thickened by fibrous tissue formation, in some places by nonstriated muscle fibers and in places by a hyaline material apparently derived from the increased connective and elastic tissue. The pulmonary arteries were thickened and in places hyalinized. It was surprising to find so much thickening of the alveolar walls with so little iron pigmentation. Many of the alveoli contained marginal deposits of hyalinized exudate, which exhibited various stages of organization and in some places had the appearance of osteoid tissue. Nowhere was calcification observed independent of bone formation.

The bone appeared in two forms—as flat plaques in the pleura (fig. 2) and as branching deposits within the lung proper (fig. 3). The latter seemed to be both interstitial and intra-alveolar. In either case the bone consisted of lamellas of bone with abundant bone corpuscles but as far as could be found, no marrow formation.

The fibrosis of the lung was different from that seen ordinarily in chronic passive congestion. First, there was the extremely sparse deposition of iron pigment and the rather focal distribution of the connective tissue increase, for there were areas that seemed relatively free from it. Second, there were organizing fibrinoid deposits within the alveoli and thickening and hyalinization of the interstitial connective tissue. The appearance was that of progressive chronic interstitial pneumonitis with ossification. Were it not that in nearly all the cases of ossification of this type there has been associated advanced chronic cardiac decompensation, it would seem as if the decompensation had nothing to do with it. However, such ossification has not been observed in uncomplicated cases of chronic interstitial pneumonitis and very rarely in cases of chronic pulmonary congestion, even when this was associated

Fig. 1.—Roentgenogram of the right lung showing disseminated ossified flecks throughout, although chiefly in the lower lobe. The left lung showed practically the same picture.

with pneumonitis. The possibility remains that the conditions favorable for ossification in chronic interstitial pneumonia are present only when pulmonary congestion is added to the picture. There is considerable evidence that venous stasis or general circulatory impairment favors bone formation provided that other conditions conducive to ossification are present.[6]

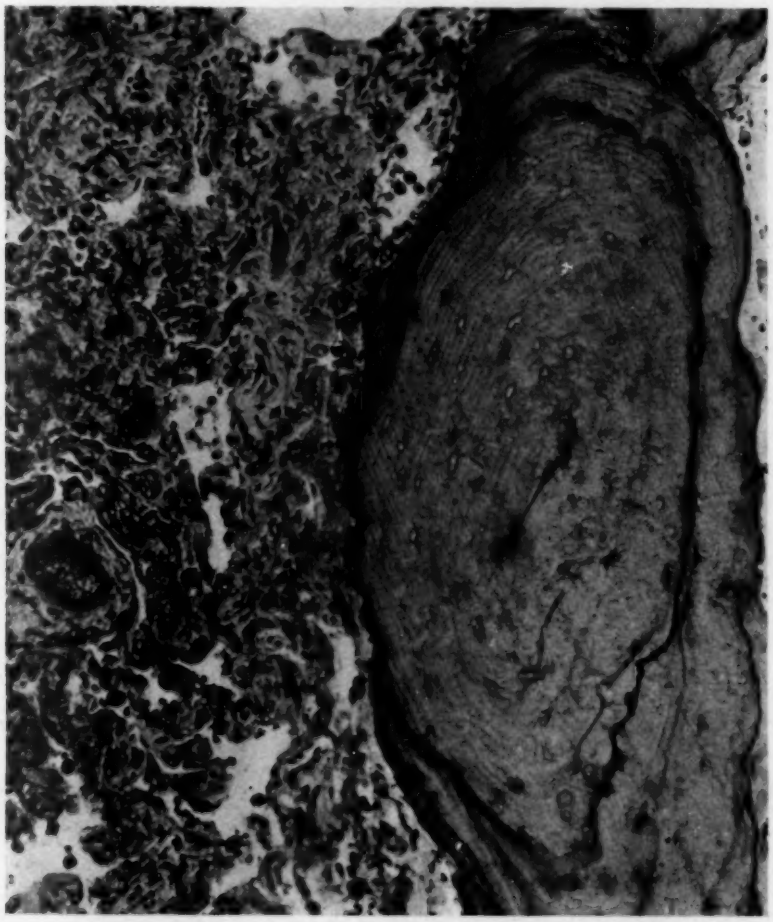

Fig. 2.—Ossified area beneath the pleura, without marrow formation (× 155). The underlying lung tissue shows chronic interstitial pneumonitis.

The findings in roentgen ray pneumonitis are remarkably similar to those seen in this case, especially the hyaline intra-alveolar membrane,[7] but in none of the human cases described in the literature is there mention

6. Roome, N. W., and McMaster, P. E.: Arch. Surg. **29**:54, 1934. Asami, G., and Dock, W.: J. Exper. Med. **32**:745, 1920.

7. Warren, S., and Spencer, J.: Am. J. Roentgenol. **43**:682, 1940.

of ossification occurring in this condition. On the other hand, roentgen ray pneumonitis experimentally produced in rabbits by Engelstad [8] led to ossification quite similar to that observed with mitral stenosis. He said, "At least half the animals (whose lungs were) examined two months or more after irradiation showed more or less widespread ossification in the form of small disseminated particles of bone," and his photomicrographs

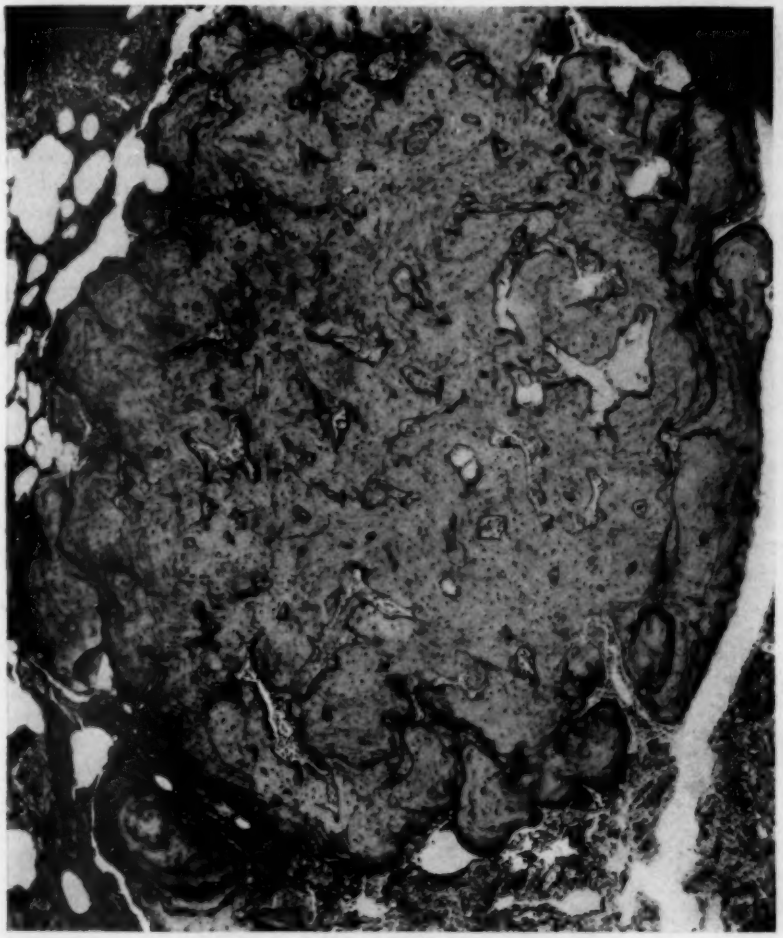

Fig. 3.—Area of branching ossification within the lung proper (× 45). Note the absence of marrow formation or of calcification independent of ossification.

resemble the human material in the cases of ossification associated with mitral stenosis. The susceptibility of the rabbit to calcification is well known [9] and is explained by the high blood calcium, Bourne and Camp-

8. Engelstad, R. B.: Acta radiol., 1934, supp. 19.

9. Wells, H. G.; Holmes, H. F., and Henry, G. R.: J. M. Research **20**:373, 1911.

bell [10] having found the normal figure to be from 12.4 to 18.6 mg. per hundred cubic centimeters. We cannot find an increase in calcium to be a constant occurrence in chronic passive congestion of the lungs, but it might be that in occasional cases the rise in carbon dioxide is responsible for an increase in blood calcium.

SUMMARY

About 45 cases of diffuse ossification of the lung unassociated with tuberculous scars have been reported. In the majority the ossifying process was of a branching type. The patients were almost exclusively aged men. About 7 verified cases of a circumscribed, nodular type, characterized by formation of discrete small flecks of bone throughout the lung, have been described. The patients were relatively young persons with mitral stenosis. To this group of cases we have added a typical example.

University of Chicago.
Harvard Medical School.

10. Bourne, M. C., and Campbell, D. A.: Biochem. J. **26**:183, 1932.

CANDIDA ALBICANS INFECTION CONFUSED WITH TUBERCULOSIS

J. B. Miale, M.D., Rochester, N, Y.

Although Candida (Monilia) not infrequently attacks various tissues in the human body, such as the skin, the mucous membranes and the respiratory tract, involvement of the central nervous system is extremely rare. A careful search of the literature reveals only a single previously reported case in which the fungus was definitely studied and identified as Monilia albicans. This was reported by Smith and Sano [1] in 1933. These authors were also unable to find reference to any previously reported case. The case to be presented would therefore appear to be the second in the literature. Other features of interest are the accompanying stomatitis, laryngitis and ophthalmitis due to Candida. During life the lesions were confused with those of tuberculosis.

Meningitis due to pathogenic fungi is not a rarity. Since Stoddard and Cutler [2] reported 4 cases of meningitis due to Torula histolytica, there have appeared scattered reports from various regions of the United States and other parts of the world, such as those of Pierson,[3] Evans,[4] Sheppe,[5] Shapiro and Neal,[6] Levin [7] and Ball.[8] Freeman [9] mentioned a case of meningitis caused by Saccharomyces and reviewed 19 other cases of various causation. Anderson [10] reported 4 cases of mycosis of the brain, 1 of which he attributed to Saccharomyces, 2 to Torula and 1 to Coccidioides. Rewbridge, Dodge and Ayers,[11] reported a case due to Endomyces capsulatus (new species). Blastomycosis of the brain has been reported by Swift and Bull,[12] Badham,[13] Gaspar,[14]

From the Department of Pathology of the University of Rochester School of Medicine and Dentistry.

1. Smith, L. W., and Sano, M. E.: J. Infect. Dis. **53**:187, 1933.

2. Stoddard, J. L., and Cutler, E. C.: Torula Infection in Man, Monograph 6, Rockefeller Institute for Medical Research, 1916.

3. Pierson, P. H.: J. A. M. A. **69**: 2179, 1917.

4. Evans, N.: California State J. Med. **20**:383, 1922.

5. Sheppe, W. W.: Am. J. M. Sc. **167**:91, 1924.

6. Shapiro, L. L., and Neal, J. B.: J. A. M. A. **81**:212, 1923.

7. Levin, E. A.: Arch. Int. Med. **59**: 667, 1937.

8. Ball, H. A.: California & West. Med. **32**:338, 1930.

9. Freeman, W.: Ann. Int. Med. **6**: 595, 1932; J. f. Psychol. u. Neurol. **43**: 231, 1931.

10. Anderson, G. C.: Arch. Surg. **42**:379, 1941.

11. Rewbridge, A. G.; Dodge, C. W., and Ayers, T. T.: Am. J. Path. **5**:349, 1929.

12. Swift, H., and Bull, L. B.: M. J. Australia **2**:265, 1917.

13. Badham, C.: M. J. Australia **2**:385, 1922. Cabot Case 25292, New England J. Med. **221**:111, 1939.

14. Gaspar, I.: Arch. Neurol. & Psychiat. **22**:475, 1929.

Moore [15] and Wilhelmj.[16] Coccidioides infection of the spinal cord was reported by Rand.[17] Hyslop[18] reported a case of sporotrichotic meningitis. Actinomycosis of the meninges has been reported by Bell[19] and Moersch.[20] There is a recent report of endocarditis due to Candida by Wikler and associates.[21] In addition, there are occasional scattered reports of yeast-caused meningitis in which the fungus was so inadequately studied as to render the report incomplete.

REPORT OF A CASE

The patient was a 30 year old white man. The history dates back to 1933, at which time the patient noted small white spots in the mouth and on the tongue. Following tonsillectomy in 1934 these became red, bleeding and painful, particularly after he had eaten hard, dry foods. The condition became progressively worse, and in August 1936 he was referred to the State Institute for the Study of Malignant Disease, in Buffalo. A biopsy specimen was taken from the mouth, and the following report was rendered: "This is tuberculosis of the submucous area. The surface epithelium is markedly hyperplastic and thickened. Typical tubercles are found below it."

In September 1936 he was seen in the outpatient department of the Mount Morris Tuberculosis Hospital, Mount Morris, N. Y., at which time he complained of the oral lesions and of an occasional cough, sore throat, sense of fulness in the throat and easy fatigability. Examination revealed nothing except for the lesion of the mouth. This was described as a chronic granulating process involving the buccal surfaces of the mouth, the inner surfaces of the lips and the surfaces of hard palate, soft palate, uvula, anterior pillars and epiglottis. The surfaces showed heaping up, induration, fissuring and a fine granularity with some injection but no bleeding. The roentgen picture of the chest showed no pathologic change. Sputum revealed no acid-fast organisms. A serum complement fixation test for tuberculosis was negative. A tuberculin test with 0.005 mg. of purified protein derivative was positive. The Wassermann reaction of the blood was negative. The patient was given a course of generalized treatment with ultraviolet rays. However, because of the development of iritis and uveitis, this therapy was discontinued until such time as the ocular condition improved.

The patient was then admitted to the eye service of the Strong Memorial Hospital, in Rochester, N. Y., in February 1937. At that time he stated that one month after the start of the ultraviolet ray treatment he noticed spots and blurring of vision in the right eye, followed rapidly by severe pain and redness. At the time of admission there was total loss of vision in the right eye.

The past history was essentially without bearing on his condition. He was working in a restaurant when the oral lesions first developed, and there were no contacts with persons known to be tuberculous.

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15. Moore, J. T.: Surg., Gynec. & Obst. **31**:590, 1920.

16. Wilhelmj, C. M.: Am. J. M. Sc. **169**:712, 1925.

17. Rand, C. W.: Arch. Neurol. & Psychiat. **23**:502, 1930.

18. Hyslop, G. E.; Heal, J. B.; Kraus, W. M., and Hillman, O.: Am. J. M. Sc. **172**:726, 1926.

19. Bell, H. H.: J. Infect. Dis. **30**:99, 1922.

20. Moersch, F. P.: Arch. Neurol. & Psychiat. **7**:745, 1922.

21. Wikler, A.; Williams, E. G.; Douglas, E. D., and Emmons, C. W.: J. A. M. A. **119**:333, 1942.

The following positive physical findings were recorded: There were enlarged, nontender cervical nodes. The left eye was normal except for internal strabismus. The right eye showed chemosis and edema of the bulbar and palpebral conjunctiva. The eyeball was somewhat shrunken, soft and tender. The cornea was opaque, with vascularization and small hemorrhagic areas in the region of the pupil. The lips were dry and fissured, and at the corners of the mouth there were deep fissures with heaped up, coarsely granular edges which merged into the buccal surfaces presenting the same thick, gray, granular appearance. The dorsum of the tongue was similar in appearance. There were a few hemorrhagic areas.

The clinical impressions were: panophthalmitis on the right, probably tuberculous; tuberculosis of the mouth.

The right eye was enucleated. Sections were interpreted as showing an acute and chronic granulomatous lesion. The inflammatory reaction involved all coats. Acid-fast stains of the sections were negative for tubercle bacilli.

A week later a biopsy specimen was taken from the inner surface of the upper lip. This was also reported as showing nonspecific granuloma. Because the patient complained of hoarseness, he was seen by a consultant on diseases of the nose and throat. The epiglottis showed the same type of lesions as the mouth. The arytenoids and ventricular bands were swollen and slightly red. The impression was that the lesions were probably tuberculous.

Laboratory tests were essentially negative. Direct smears and inoculations of guinea pigs with material from the mouth were negative for acid-fast organisms. Sputum was also free from such organisms on smear and on inoculation of guinea pigs.

The man was discharged and was seen at long intervals in the ophthalmologic outpatient department for glasses and a routine follow-up. From March 1937 to September 1941 his condition was apparently unchanged.

Nov. 27, 1941, he was brought in stuporous and lethargic and unable to give a clear history. The available information indicated that he had suffered from very severe frontal headaches for the previous two months. These were worse at night. For the previous two weeks there had been marked lethargy, and the patient was unable to respond to spoken words. He was getting weaker rapidly and had fever and generalized muscular pains.

His temperature was 38.2 C. (100.8 F.), pulse rate 86 and respiratory rate 24; his blood pressure was 106 systolic and 70 diastolic. The following pertinent findings were noted: The patient was a 30 year old man, was very lethargic and markedly emaciated, appearing acutely and chronically ill. There was no generalized adenopathy. The teeth were foul and in bad repair. The tongue was foul and dry, covered with whitish exudate and deeply fissured. The entire buccal mucosa also was covered by a thick cheeselike material. The patient was drowsy and lethargic, the mentality was clouded, and the neck was questionably stiff. The left pupil was fixed and did not react to light. The left fundus showed choking of the disk. Other cranial nerves were intact. There were involuntary muscular contractions and fibrillary twitchings of the extremities. The deep reflexes were all absent. The Babinski reflex was equivocal.

The Wassermann reaction of the blood was negative. The red blood cell count was 4,950,000. The hemoglobin level was 15.0 Gm. The white cell count was 11,600 (neutrophils 83 per cent, lymphocytes 15 per cent, monocytes 1 per cent, eosinophils 1 per cent). The urine was normal. The spinal fluid showed an initial pressure of 250 mm. of water and a final pressure of 200 mm. The Pandy test was positive, and there were 117 cells without and 109 with acetic acid. Most of the cells in the spinal fluid were described as mononuclears. The spinal fluid

sugar was too low to read. The spinal fluid protein was 195 mg. per hundred cubic centimeters, the colloid gold curve was 2555554432, the Wassermann reaction was negative, and a smear was negative for acid-fast organisms. Examination of the spinal fluid four days later showed 122 cells, sugar too low to read, protein 150 mg. and chlorides 114 milliequivalents. Two days later the spinal fluid pressure rose to 400 mm. Spinal fluid cultures and smears were negative. A guinea pig inoculated with the fluid showed no tuberculosis. Repeated smears of exudate from the mouth were negative for acid-fast organisms.

The temperature, which on admission was 39 C. (102.2 F.), dropped to normal in the next five days, and then began to rise gradually until terminally it measured 41 C. (105.8 F.). No therapy was given other than supportive measures. The patient remained somewhat somnolent and stuporous but responded to painful and auditory stimuli. He would not eat and was maintained on parenteral fluids and dextrose. The neurologic picture remained unchanged. He became more and more lethargic and less responsive to stimuli. Terminally, he was comatose and extremely debilitated, and he died on Dec. 19, 1941, after a stay in the hospital of twenty-three days and a total illness of approximately eighty-three days.

The diagnosis at discharge was questionable tuberculous meningoencephalitis.

Autopsy.—The anatomic diagnosis was: chronic stomatitis, glossitis and laryngitis due to Candida (Monilia) albicans; meningitis and ependymitis due to C. albicans; granulomatous endarteritis of the superior cerebellar artery; old enucleation of the right eye (ophthalmitis due to C. albicans); bronchopneumonia; emaciation.

A complete autopsy was done, and all organs were carefully studied, but only those showing gross pathologic change are mentioned here.

The body was that of an extremely emaciated 30 year old white man. The right eye was absent. The lower lip had a deep ulcerated surface which extended into the mouth and was bright red, slightly granular, dry and sharply demarcated. All surfaces of the mouth were thick and granular and covered with abundant gray exudate. No cervical glands were palpated.

The lungs weighed 420 Gm. each. Both lower lobes were reddish brown and on section were moist. The cut section presented a few scattered small granular areas which were slightly firmer in consistency, grayish and bulging.

The trachea and the larynx were normal in their external configuration. When the larynx was opened, the mucosa appeared pink and glistening except in the region of the left vocal fold and ventricle. In this region the surface was granular and gray. There were no areas of ulceration, although in the posterior angle of the ventricular fold the mucosa appeared soft, edematous and reddish brown. The granular areas extended up into both ventricular appendixes and onto the posterior surface of the epiglottis. The tongue showed a markedly thickened and irregular surface, which was nodular, gray and dry. There was no gross ulceration.

The brain weighed 1,370 Gm. The dura was normal. The venous sinuses were normal. At the tip of the left parietal lobe was one small area in the pia-arachnoid which was very finely granular and grayish, consisting of many minute glistening sandy nodules which were slightly opaque. Over the right parietal lobe were seen a few small sandy nodules presenting the same appearance. These small tubercle-like structures measured only a fraction of a millimeter in diameter and were distributed about the pial vessels. The base of the brain presented a thick, grayish yellow, stringy exudate over the pons and peduncles, extending into the fissures between the temporal and frontal lobes and encircling the brain stem posteriorly. In this thick exudate were seen a few small raised tubercle-like nodules. The exudate did not involve the superior aspect of the cerebral hemispheres.

The brain was cut after fixation. The cortex was everywhere of normal thickness and showed no abnormal areas. The white substance presented no areas of softening. The lateral ventricles were of normal size, but along their entire length the ependymal surface was covered with thick, irregular nodular masses which

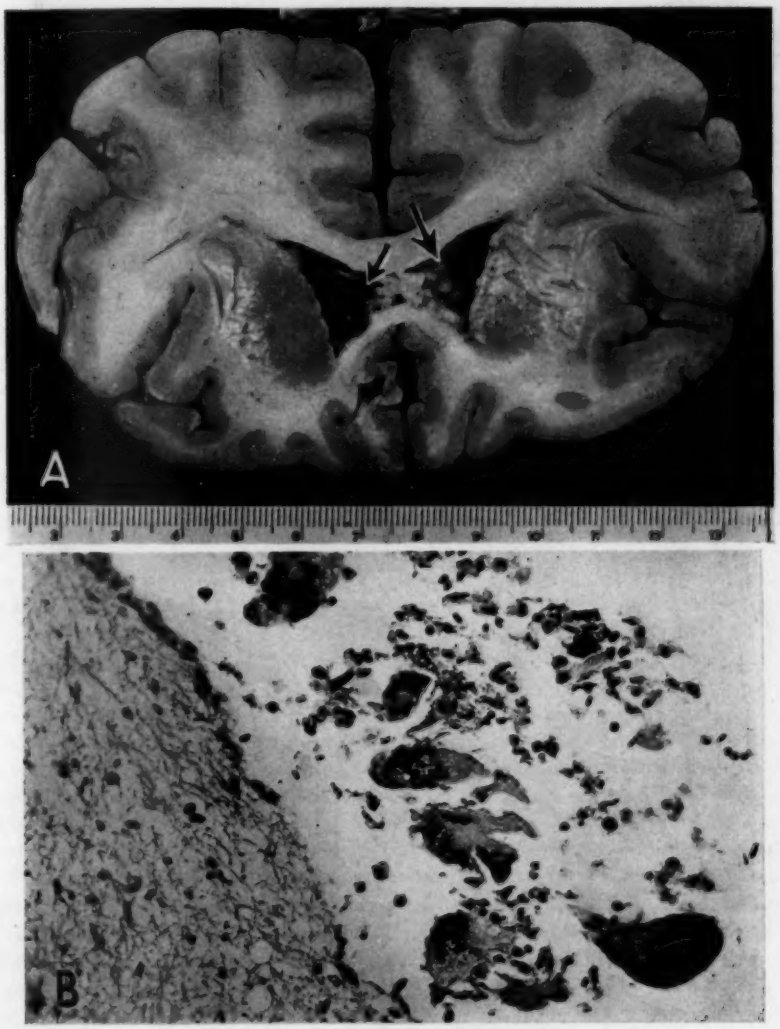

Fig. 1.—*A,* coronal section of the brain through the anterior portions of the lateral ventricles. The thick masses of Candida (Monilia) proliferating on the ependymal surfaces can be seen as glistening nodules projecting into the lumen. All ventricles showed this type of involvement. *B,* material from the lateral ventricle. This illustrates the large multinucleated giant cells seen in all portions of the exudate. Blastospores can be seen in at least two of the giant cells. This is an important feature of fungous lesions and may be useful in differentiating them from the lesions of tuberculosis. Gram stain; × 210.

projected into the cavity. These were grayish white, glistening and translucent and seemed to be firmly attached to the surface although they were, of themselves, friable in consistency. The exudate measured about 8 mm. at its greatest thickness. The third ventricle was slightly dilated, and the ependymal surface was covered with the same kind of granular translucent exudate. The pineal gland appeared large and cystic, measuring 8 by 5 mm. The aqueduct was normal in size but occluded by the same material as that seen in the ventricles. The fourth ventricle showed the same process to a less degree. The medulla and the cerebellum were normal.

Histologic Studies.—Sections of all organs were taken routinely. Only the significant ones are described. All blocks were fixed in Zenker's formaldehyde solution [22] and embedded in paraffin.

The lungs revealed marked congestion with extravasated blood in the alveoli. There were scattered patches of bronchopneumonia. No epithelioid cells, caseation or tubercle formation was seen. The routine stains showed no fungous elements. Many clumps of bacteria were present. Special stains were ngeative for fungi.

Sections of the tongue and of the larynx were studied with the routine hematoxylin-eosin stain, Mallory's phloxine–methylene blue stain, MacCallum's modification of Goodpasture's Gram stain, and a stain for acid-fast organisms. The sections stained with the routine hematoxylin and eosin showed the lesions to be granulomatous in character. There was ulceration of the epithelium, with marked round cell infiltration, tubercle formation and the presence of many epithelioid cells and giant cells. A few mycelial threads could be identified, although they stained faintly. These were present in the ulcerated areas as well as in the deep tissues. In the larynx the tubercles were composed of a necrotic center and a ring of numerous multinucleated giant cells. In the caseous area a few mycelial threads were identified. There was hyperplasia of the epithelium where this was not ulcerated. The round cell infiltration was most marked in the areas of ulceration, but was also present throughout the deeper tissues (fig. 2 *B*).

The Gram stain brought out the fungous elements so that they could be identified with ease. They were present not only in the ulcerated areas but also in the areas of deep necrosis and in the centers of the tubercles. Both mycelial threads and blastospores could be identified. The mycelia were typical and were seen as slender rods a few microns thick, the walls of which stained darkly and had an internal structure which varied somewhat (fig. 2 *A*). Most typical were condensations of gram-positive material which gave them an irregularly segmented appearance and which outlined the clear oval spaces, giving the mycelia a beaded appearance. These clear spaces stained with the usual fat stains. The blastospores were easily identified when they occurred in clusters, but this formation was not usual, and the individual cells were difficult to find. They appeared as small ovoid cells about 4 microns in diameter, staining darkly gram-positive and showing a thin, doubly refractile capsule which took the counterstain poorly.

The phloxine–methylene blue stain made the fungous elements well visible but was less useful as a differential stain because, although it allowed ready identification of the mycelia, the blastospores were not easily differentiated from lymphocytes. The stain for acid-fast organisms showed that the mycelia were not acid fast, and no acid-fast organisms were found.

In view of the findings in the autopsy sections, the biopsy specimens taken from the mouth on Feb. 4, 1937 were recut and stained with the differential stains

22. This is Zenker's stock solution with addition of solution of formaldehyde.

already described. The hematoxylin-eosin stain showed a granulomatous lesion with tubercle formation and both giant cells and epithelioid cells. The Gram stain revealed typical fungous elements in the deep tissues and in the areas of caseation.

The pathologic picture in the brain sections related to the meningeal and the ependymal reactions.

The basilar meninges showed extensive involvement. They were thickened and presented a thick exudate of lymphocytes, with scattered plasma cells, monocytes

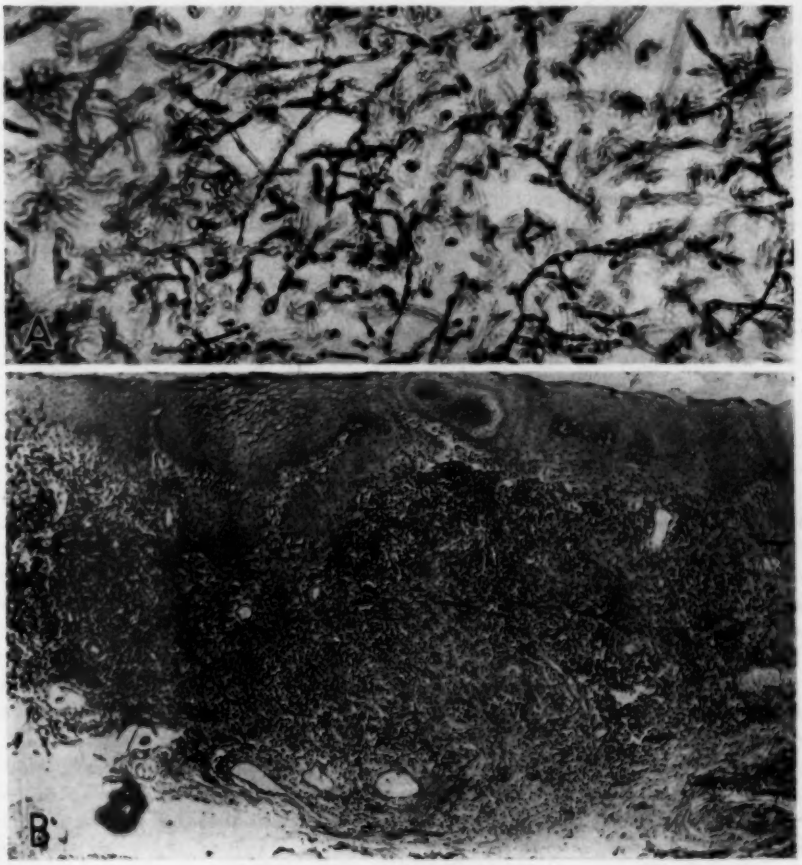

Fig. 3.—*A*, high power view of a large field of mycelia from one of the brain sections. The branching and the typical segmented and beaded appearance are characteristic. The clear vacuoles in the mycelia are also typical. Blastospores are present but are not easily identified. Gram stain; × 430. *B*, section from the larynx showing the granulomatous appearance of the lesion. There is marked round cell infiltration throughout, and deep in the section can be seen a large tubercle. In the center of the necrosis slender gram-positive mycelia can be identified even under this magnification. Gram stain; × 60.

and large phagocytic cells. Only an occasional polymorphonuclear leukocyte was seen. The meningeal reaction was more intense in the deep recesses of the sulci,

and in several areas large masses of mycelia were seen. Many large giant cells were seen around such areas, and many round cells were present about the margins. With special stains, the mycelia presented the typical appearance already described. Some of the cells interpreted as being lymphocytes in the sections stained with hematoxylin and eosin were seen to be typical blastospores.

The ependymal reaction was striking and unusual. Sections through the ventricles showed that the large translucent grayish white masses seen grossly were actually huge collections of fungous elements proliferating within the ventricles (fig. 1 *A*). One of these masses was almost 1 cm. in diameter. The ependymal surface was replaced by the granulomatous tissue composed of giant cells and chronic inflammatory cells. The giant cells occasionally contained clear ovoid areas the size of one of the nuclei, and with the Gram stain these were shown to be ingested blastospores (fig. 1 *B*). The mycelia presented a typical appearance. Blastospores were not as numerous as mycelial threads, although they were also present in large numbers.

The third and fourth ventricles were almost completely occluded by masses of mycelia. The pineal body was seen completely surrounded by mycelia and showed calcification and degeneration. Its center was cystic and was filled with a clear pink-staining colloid-like material.

The vascular system of the brain had been involved in the process. There was perivascular infiltration of the smaller vessels in the brain substance, and the cells were all mononuclears. The superior cerebellar artery was seen in cross section between the cerebellum and the pons and presented an unusual picture. The lumen was markedly narrowed by very marked thickening and proliferation of the intima. This showed degeneration of its fibers, vascularity and infiltration by lymphocytes, monocytes and plasma cells, with a large number of epithelioid cells. At the junction of the intima and media there was a separation of the two coats, and in the outer intima were many giant cells with numerous nuclei. Scattered among them were a few mycelial threads. The adventitia showed moderate mononuclear infiltration and a thick, markedly infiltrated zone which formed a complete collar around the artery. Here, again, there were great numbers of round cells, and both mycelia and blastospores were found. Other arteries showed marked perivascular reaction, but the intima was not involved.

The cerebellum showed one area of degeneration of the granular layer with loss of cells. There was no glial reaction. The Purkinje cells appeared to have been spared.

The right eye, which was enucleated in 1937, was restudied, and special stains were used. The appearance with the routine stains has been described elsewhere. The differential stains showed that in the main bulk of the exudate and necrotic tissue obliterating the posterior chamber and vitreous there was a large aggregate of typical mycelia.

Isolation and Identification of the Fungus.—The basilar exudate from the brain was sampled by sterile technic and streaked on standard Sabouraud agar plates. Direct smears prepared with acid-fast stains were found to be negative for acid-fast organisms.

The Sabouraud agar plates showed pure colonies, which appeared after standard incubation for twenty-four hours at 37.0 C. At first these colonies were very small, dull, grayish white and flat with smooth surfaces. Later they coalesced to form large creamy colonies with a fringe of mycelia around the margin growing radially into the medium. Among old cultures the larger colonies showed raised and puckered central areas. Smear preparations from young colonies showed gram-positive blastospores and few mycelia. The spores were gram positive, about 4 or 5

microns in diameter and oval and had thin, doubly refractile capsules. The mycelia showed occasional branching with terminal spore formation.

Young colonies from the Sabouraud agar plates were subcultured on fresh Sabouraud agar plates and on blood agar plates. On blood agar pure colonies were isolated, which appeared after forty-eight hours at 37.0 C. These were small uniform round grayish colonies with a smooth surface and edge. Further incubation produced no changes other than growth in size. The subcultures on Sabouraud agar plates showed no change in colony characteristics or in the appearance of the smears prepared from them.

Material from the growth on the original Sabouraud agar plates was suspended in saline solution and injected intraperitoneally into white mice. The mice remained alive and well for many weeks after the injection of the suspension.

On the basis of these preliminary studies it was decided that the etiologic agent was a fungus, and that this belonged in the genus Candida (Monilia).

Dr. N. F. Conant made the final identification. A culture was sent to him, and by following the procedure advocated by Martin and co-workers [23] he identified the fungus as Candida albicans. Details of the method will be found in the references cited, but briefly the identification includes the character of the growth on various mediums, fermentation of sugars and pathogenicity tests. This organism is pathogenic for rabbits.

COMMENT

This case presents many features of interest both to the pathologist and to the clinician. Clinically, the diagnosis of tuberculous meningitis was made. This was a likely diagnosis in view of the reported tuberculosis of the mouth, although biopsy specimens were always reported from this laboratory as showing nonspecific granuloma. At no time were acid-fast organisms demonstrated in the exudate from the mouth, in biopsy sections or in the spinal fluid. The cells in the spinal fluid were described as mononuclear cells, and it is possible that some of these may have been yeast cells. In the fresh state, and in the absence of a suspicion of any fungus, yeast cells may easily be mistaken for small lymphocytes if no attention is paid to the distinctive doubly refractile capsule. This point has been stressed by many writers on fungous diseases. A careful search must be made for fungous elements if the correct diagnosis is to be made.

Other laboratory findings are not helpful in making the diagnosis. The spinal fluid sugar in this case was always reported as too low to be read, and this finding supported the diagnosis of tuberculous meningitis. It is of interest to note the findings in other cases. Smith and Sano [1] did not give spinal fluid sugar levels in their case. They reported the ependymal surfaces as clear. Freeman [9] reported on 19 cases of fungous meningitis but stated only that "chemical studies on spinal fluid are negative." In the case of sporotrichotic meningitis reported by Hyslop and associates [18] the values for spinal fluid sugar ranged from 42 to 88 mg. per hundred cubic centimeters. Anderson [10] reported 4 cases; in 2 the spinal fluid sugar amounted to 5 to 10 mg. per hundred cubic centimeters; in 1 instance the value was not reported,

23. (*a*) Martin, D. S.; Jones, C. P.; Yao, K. F., and Lee, L. E., Jr.: J. Bact. **34**:99, 1937. (*b*) Martin, D. S., and Jones, C. P.: ibid. **39**:609, 1940.

and in the last it was 93 mg. In the case reported by Rewbridge and associates [11] the spinal fluid sugar amounted to 36 mg. Therefore, the sugar level may be reduced moderately or markedly, but no differential information can be obtained.

Candida infections of the mouth have been reported many times, as in the papers by Zeisler,[24] Engman and Weiss,[25] Frost and co-workers [26] and Smith.[27] They are common in childhood and are frequently seen in old people with chronic debilitating diseases. Their importance is that in the older age group they may be mistaken for tuberculosis, as in the case reported here, or for some other type of granuloma. The pathologic picture is that of granuloma, and in such cases the pathologist should attempt to demonstrate a specific etiologic agent by means of acid-fast stains, cultures, Gram stains and serologic tests. Before the diagnosis of nonspecific granuloma is made, fungous infection should be ruled out by means of differential stains.

The same applies to laryngeal lesions, although fungous infections in this region are not common. Clerf and Bucher [28] reported 3 cases of laryngeal moniliasis and collected 6 more from the literature. Here, again, the fungous lesion must be distinguished from other types of granuloma.

Ophthalmitis of fungous origin is extremely rare, and there has probably been no previous report of an infection due to Candida. However, because the literature is not recent, the nomenclature used for the implicated fungi is very confused, and it is impossible to be sure.

The pathologic appearance of fungous lesions is interesting. A granuloma is represented, and the lesion may be mistaken for tuberculosis by expert pathologists. Giant cells are numerous, probably more numerous than in tuberculosis, and definitely more numerous than in the usual syphilitic lesion. They often contain ingested blastospores and mycelial elements, which is an important differential point. Tubercle formation may be typical, and necrosis may be a feature. Without recognizing the fungous elements by special stains, the lesions may easily pass for tuberculosis. Candida spores are difficult to identify, even with special stains, but some of the other fungi, such as Coccidioides and Blastomyces, are more easily recognized because the spores are larger and have a thick doubly refractile capsule.

The diagnosis of a gross specimen is equally difficult. The distribution of the meningitis in this case was like that typically seen in tuberculosis, and the definite diagnosis hinged on isolation of the fungus by cultural methods and on finding the fungous elements in the histologic sections.

Classification of pathogenic fungi is a matter for experts in that field, and in this case I have been guided by Dr. Conant's advice in calling this organism Candida albicans. At an informal meeting of medical

24. Zeisler, E. P.: Arch. Dermat. & Syph. **15**:171, 1927.

25. Engman, M. F., and Weiss, R. S.: Arch. Dermat. & Syph. **1**:119, 1920.

26. Frost, K.; Sutherland-Campbell, H., and Plunkett, O. A.: Arch. Dermat. & Syph. **20**:811, 1929.

27. Smith, E. C.: J. Trop. Med. **31**:101, 1928.

28. Clerf, L. H., and Bucher, C. J.: Ann. Otol., Rhin. & Laryng. **45**:923. 1936.

mycologists at the Third International Congress for Microbiology in September 1939, it was agreed to substitute the generic name of Candida for Monilia, pending official action by the rules committee of the International Botanical Congress (Martin and Jones [23b]).

SUMMARY

A case of Candida albicans infection is reported which during long periods of careful study in several clinics was considered to be a tuberculous infection. It is probable that this condition is not as rare as a study of the literature would indicate.

Mycosis should be considered in all cases of apparently nonspecific granuloma. In such cases Gram's stain is very useful in identifying the fungous elements in tissue sections.

A careful review of the literature reveals only 1 previously reported case of meningitis due to Candida albicans.

General Reviews

THE BLOOD CHOLESTEROL

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I. NORMAL PHYSIOLOGIC VARIATIONS

Though cholesterol is found in virtually all tissues of vertebrates and invertebrates, its pathways of absorption, synthesis, destruction and excretion are unknown, as is also its part in the structure and the metabolism of living cells. It is not a foodstuff in the ordinary sense, yet its concentration in the circulating blood is of the same order as that of sugar and of fats. From a teleologic point of view, therefore, it is a substance of great physiologic significance. On the basis of its physical properties it is classified with the lipids, though chemically there is no relation. In chemical structure it resembles rather the terpenoid substance of plant origin. Indeed, the view was once held that cholesterol came exclusively from plants, and that the animal possessed no power to synthesize or to destroy it. Investigators know now, however, that it can be readily metabolized; and though nothing is known of its function in the organism, its chemical relatives, the bile acids and the adrenal and the androgenic and the estrogenic hormones, play a part in the most fundamental of the life processes.

The relationship between the blood cholesterol level and disease has been the subject of much clinical and experimental study, and an overwhelming amount of information has accumulated. Unfortunately, many conclusions which have been drawn rest on mistaken or confused ideas of what are normal cholesterol levels. The first part of this review will be devoted, therefore, to normal physiologic variations of the blood cholesterol. With this knowledge as a background, the metabolism of cholesterol and its relation to pathologic conditions will constitute the second and third parts of this review.

METHODS OF DETERMINING CHOLESTEROL

Before discussing the blood cholesterol and its variations, a few words regarding its determination are necessary. Of the many color reactions given by sterols (Sobotka), the most popular is the Liebermann-Burchard reaction (Lieber-

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mann), utilizing the color developed by cholesterol in the presence of acetic anhydride and concentrated sulfuric acid. This reaction forms the basis of the quantitative methods of Grigaut (1910), Bloor (1916 a), Autentrieth and Funk and their innumerable modifications.

There are two serious objections to direct colorimetric estimation. First, an appreciable difference exists between free and bound cholesterol in the amount and velocity of the color development.[1] Second, the studies of Gardner and Williams, Yasuda, Reinhold, and Kelsey have shown that cholesterol esters yield values 10 to 30 per cent higher than the equivalent amounts of free cholesterol. Another source of error in the direct colorimetric procedure is the presence of interfering colors in the lipid extract and the possibility that other substances besides cholesterol in blood may contribute to the color (Reinhold).

For the precise determination of cholesterol, a method based on precipitation with digitonin is requisite. This essentially involves extraction of cholesterol and its esters by means of an organic solvent or mixture of solvents and precipitation of the free cholesterol before and after saponification by means of digitonin. The cholesterol content of the digitonide thus formed may be determined gravimetrically,[2] oxidimetrically [3] or colorimetrically.[4] The success of the gravimetric and oxidimetric methods rests on the assumption that the precipitated digitonide has a constant composition and that substances other than sterols are not precipitated. The first assumption is correct only if the factor is determined on each batch of digitonin used, as Schoenheimer and Dam found that the composition of the precipitate varies with the source of the digitonin. The second assumption requires qualification. Inasmuch as digitonin precipitates saturated sterols as well as cholesterol, the total sterol content rather than the latter is determined in these methods. Saturated sterols comprise only 1 to 5 per cent of the total sterols of body tissues (Schoenheimer, von Behring and Hummel); for all practical purposes, therefore, the total sterol and cholesterol may be considered identical. Other substances, particularly some of the steroid hormones, also are precipitated by digitonin. Though their concentrations in blood are negligible in comparison with that of cholesterol, they may amount to an appreciable fraction of the unsaponifiable matter of certain organs.

The recently reported method of Schoenheimer and Sperry has deservedly achieved popularity. In this procedure the digitonide after precipitation and subsequent removal of interfering substances is redissolved in acetic acid and the cholesterol determined colorimetrically by means of the Liebermann-Burchard reaction. Though originally adapted for the Step photometer, the color may be read conveniently by means of the photoelectric colorimeter. As digitonin does not produce an interfering color under the conditions of the method, the objections to the gravimetric and oxidimetric procedures are avoided, and as the cholesterol is free from other possible chromogenic substances and is determined only in the free state, the objections to the direct colorimetric procedure do not apply.

Another source of error in determinations of cholesterol is in the method used for its extraction. Those methods which involve preliminary drying of the blood or tissue by heating or by mixture with anhydrous chemicals, such as plaster of paris or sodium sulfate, are unsatisfactory (Gardner and Fox, 1924). Hot alcohol

1. Mueller. Page and Rudy.
2. Ewert, 1933. Man and Peters, 1933. von Szent Györgyi. Windaus.
3. Boyd, 1933 a. Kirk, Page and Van Slyke. Okey, 1928.
4. Kelsey. Schoenheimer and Sperry.

apparently is the best extracting agent for cholesterol and other lipids, and its use is the basis of the method developed by Bloor (1928 d) for blood and tissue lipids. It has the virtues of completeness of extraction, avoidance of oxidation and simplicity. Man and Gildea (1932) reported that the method as described by Bloor, with a mixture of 3 parts of alcohol to 1 of ether being used, gave blood lipid values 5 to 31 per cent too low. Boyd (1936 d) attributed this to the use of insufficient extracting fluid. He found extraction was complete in five minutes if 25 parts of fluid to 1 part of blood were used.

In interpreting data on blood cholesterol, the matter of whether the determinations have been carried out on serum, plasma or whole blood should be considered. As the red cells contain only small amounts, if any, of cholesterol ester, and the total cholesterol content of the cells is generally lower than that of the plasma, values obtained for the whole blood will be characterized by a lower total cholesterol and an increased proportion of free cholesterol. Shope (1928) observed lower cholesterol ester values for oxalated or citrated plasma than for serum. He attributed this to partial hydrolysis of the cholesterol esters in the presence of the anticoagulant. This later more correctly was attributed to alteration of the plasma and red cell volumes brought about by the anticoagulant.[5] The resultant reduction in the concentration of the plasma may amount to as much as 15 per cent. Serum and heparinized plasma give identical values for free and total cholesterol, which more truly represent their concentrations in the noncellular portion of the blood (Sperry, 1937 i).

REPRESENTATIVE VALUES FOR BLOOD CHOLESTEROL

An overwhelming amount of clinical and experimental data regarding blood cholesterol has accumulated, and comparison is difficult when so many and such various methods of extraction and analysis have been employed. Of the thousands of reports on the blood cholesterol and its variations only a small proportion can be cited: those which in the author's opinion are of greatest significance.

Representative values for the concentration of cholesterol in the serum or the plasma of normal persons are those of Gardner and Gainsborough (1927 c) (169 and 153 mg. per hundred cubic centimeters of plasma for normal men and women, respectively, with standard deviations, respectively, of 41 and 33 mg. per hundred cubic centimeters); Okey and Boyden (149 mg. per hundred cubic centimeters, with a variation of 41 mg. for normal women); Man and Peters (1933) (207 mg. per hundred cubic centimeters, with a range from 162 to 258 mg.), and Boyd (1933 a) (162 mg. per hundred cubic centimeters, with a standard deviation of 20 mg., for normal women).

More recent studies have established higher normal average values as well as a wider range of normal variation. Kirk, Page, Lewis, Thompson and Van Slyke, in 1935, reported a study of the plasma lipids of 66 normal men ranging from 21 to 91 years. The values for

5. Gardner, Gainsborough and Murray, 1938 c. Paget and Pierrart. Sperry, 1937 i.

the concentration of total cholesterol in plasma varied from 109 to 376 mg. per hundred cubic centimeters, with a mean of 232 mg. and a standard deviation of 62 mg. Age caused no significant variation. The values were higher than those obtained previously but were confirmed in another comprehensive study by Sperry (1936 f), who with 91 normal persons of both sexes found values ranging from 131 to 392 mg. per hundred cubic centimeters of serum, with an average of 209 mg. Some increase was noted with age. These results disclose that the range of cholesterol concentration considered normal in former years must be extended. In its variability cholesterol differs significantly from most of the other constituents of the blood, which normally fluctuate within narrow limits.

Cholesterol Esters.—Cholesterol, because of its alcohol group, can form esters, and in the blood a large proportion of it is thus combined with fatty acids. Constancy in the relation between the free and the esterified cholesterol of the blood was noted first by Bürger (1928), who reported that 30 per cent of the total blood cholesterol exists in the free form. Bloor and Knudsen reported a higher value: 40 per cent. Gardner and Gainsborough (1930 a, 1927 d), however, were unable to confirm this observation, nor could Kirk and his co-workers (1935), who reported values for the amount of free in the total cholesterol ranging from 22 to 72 per cent. In contrast to Kirk's results, the equally careful and comprehensive study of Sperry (1936 f) presented an entirely different picture. Though both investigators reported approximately equal ranges for the total cholesterol, Sperry found the ratio of free to total cholesterol to be remarkably constant. The amount of free in the total cholesterol varied between the narrow limits of 24.7 and 30.1 per cent, with a standard deviation of only 1.4 per cent from the average of 26.9 per cent. The reason for these differences is not evident. The ranges for the total cholesterol being approximately equal, they could not be attributed to inadequate extraction or to the use of serum in the one study and heparinized plasma in the other, for both have been shown to yield similar values (Sperry, 1937 i).

Further evidence for the constancy of the relation between free and total cholesterol in serum has been reported by Sperry in a large group of children (Sperry, 1936 g) and in over 100 persons who died by violence and whose blood was drawn post mortem (Landé and Sperry). Several others have since confirmed the constancy of this ratio in a variety of conditions,[6] and Gardner has modified his procedure and reported more constant values for the ratio (Gardner, Gainsborough and Murray, 1938 b).

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6. Muhlbock and Kaufmann. Offenkrantz. Offenkrantz and Karshan. Smith and Marble.

Enzymes for the Synthesis and the Hydrolysis of Cholesterol Esters.—The remarkable constancy of the ratio between free and combined cholesterol in spite of variation in the total blood cholesterol indicates the existence of an enzyme system regulating their proportions in the blood. This subject only recently has been investigated intensively, with contradictory and confusing reports. Sperry (1936 e) found that esterification of free cholesterol occurred in serum incubated alone or diluted with saline solution. Esterification also proceeded when the serum was incubated with saline extracts of various tissues, though not to the extent with serum alone. Further studies (Sperry and Stoyanoff, 1937 c, d) led to the finding that the esterification occurring on incubation of dog and human serum was inhibited by bile salts. The inhibition was proportional to the concentration of the bile salts until a concentration was reached at which no esterification or hydrolysis occurred. With larger amounts than this, the proportion of free to ester cholesterol in human serum remained unchanged, but in dog serum an increase in the amount of bile salts caused complete splitting of the cholesterol esters. The greatest effect was obtained with the conjugated bile salts: taurocholate and glycocholate. Monkey serum acted like human rather than dog serum.

The enzymatic nature of this reaction was shown by the fact that no change occurred in serum heated for one hour at 55 C. (Sperry and Stoyanoff, 1938). When human or dog serum thus inactivated was incubated with unheated serum of either species, esterification occurred. In the presence of taurocholate dog serum and globulin fractions from dog serum promoted the hydrolysis of cholesterol esters in heat-inactivated human or dog serum. Human serum, on the other hand, or its globulin fraction did not promote hydrolysis of cholesterol esters in heat-inactivated serum of either species. Sperry concluded, therefore, that the esterification and the hydrolysis are independent, being catalyzed by different enzymes, both present in dog serum, but only one, the esterifying, in human serum. Riegel, Ravdin and Rose, however, in similar experiments, reported that hydrolysis occurs in both human and dog serum when these are incubated with bile salts.

EFFECT OF AGE ON VALUES

At birth the blood cholesterol is distinctly low. In regard to infants from 4 to 25 days old, Sperry (1936 g) found values for heparinized plasma that ranged from 71 to 192 mg., with an average of 133 mg., per hundred cubic centimeters; after a pronounced increase during the first four days of life, the cholesterol level remained unchanged. Constancy of the ratio of free to total cholesterol, observed in adults, was not characteristic of infants' plasma, in which the amount of com-

bined cholesterol in the total varied from 41 to 72 per cent. Muhlbock and Kaufmann obtained values in close agreement with those of Sperry. Blood serum obtained from the umbilical cord at birth contained 70 mg. of cholesterol per hundred cubic centimeters, of which about 70 per cent was in the ester form. From the first to the fourth days of postnatal life, the average increased from 91 to 137 mg.; the values then remained constant through the twelve day period of study.

There is a gradual and slight increase with age throughout childhood,[7] the average values for boys and girls being about the same.[8] Basal metabolic rates and intelligence quotients could not be correlated with blood cholesterol levels,[9] and no differences have been observed between white and Negro children (Molitch and Poliakoff). The ratio of combined to free cholesterol was fairly constant in normal children, aged 2 months to 12 years, at 2.67 ± 0.55—about the same as the adult value (Offenkrantz and Karshan).

In a study of normal men, 21 to 91 years of age, Kirk and co-workers (1935) found no significant effect of age on the levels of cholesterol and its esters or other lipids in the blood plasma, nor any change in their proportions. Muhlbock and Kaufmann, on the other hand, observed an increase in free and in combined cholesterol with age in women. The variations were from an average of 200 mg. during the third decade of life to 260 mg. during the seventh decade. Several authors claim to have found lowered cholesterol values for normal persons in extreme old age.[10] Such conclusions are open to question, however, because of the possibility of nutritional or other metabolic defects in these subjects.

EFFECT OF PERSONAL AND ENVIRONMENTAL FACTORS

The wide normal range of the concentration of cholesterol in the blood raises the question of whether this variation represents differences between persons or variations in a single person over a period of time. The consensus of reports is that the cholesterol content of the blood of an adult is constant and independent of internal and external conditions. The cholesterol content of the blood of normal women during the intermenstrual period was found by Okey and co-workers (1927, 1933) to be constant, this observation being confirmed by Muhlbock and Kaufmann (1928, 1938) and Offenkrantz. The studies of Sperry (1937 i) over periods up to twenty-eight months on 25 adults of both sexes, and of Turner and Steiner over periods of seven to

7. Baylac and Sendrail. Ward.
8. Rothbart. Ward.
9. Molitch and Poliakoff. Rothbart.
10. Brodin and others. Bürger and Möbius.

fourteen months on patients suffering from a variety of ailments, agree that the level of cholesterol remains remarkably constant for each person. The same conclusion was reached by Bloor (1933) on the basis of a study on dogs and by Boyd (1938) and Harnes (1928) in a study on rabbits. Man and Gildea (1937), on the other hand, after a study of 4 males and 6 females during periods of from three months to two years, and Schube after a study of 10 persons over a period of sixteen weeks, reported variations in the blood cholesterol level of single persons. Inspection of their data reveals that in the subjects of the former study the widest range of variance calculated as deviation from the average was only 15 per cent; in the latter, it was only slightly higher. From the widest fluctuations reported it is evident that the cholesterol level of the blood of a single person of a group is far more constant than the range of variation found in the group.

An interesting relation between the serum lipid levels and the body build has been shown by Gildea, Kahn and Man. They determined the serum lipids in men and women belonging to two distinct body types: the stocky, or heavy, pyknic, and the slender, asthenic. These subjects were normal and free from obvious endocrine disorders. They ranged in age from 18 to 50 years. The pyknic men had an average blood cholesterol concentration of 230 mg. per hundred cubic centimeters; the asthenic averaged only 168 mg. The slight difference found between the pyknic and the asthenic women, the averages being, respectively, 205 and 196 mg. per hundred cubic centimeters, was attributed in part to the difficulty in distinguishing the morphologic type in this sex. Offenkrantz confirmed these observations in unpublished data.

This relation affords an interesting field for speculation regarding the possibility of a connection between body build and susceptibility to diseases associated with abnormal deposition of cholesterol. Such speculation is useless, however, in the present state of ignorance of cholesterol metabolism, except possibly as an exercise of the imagination.

The effect of racial and climatic factors on the blood cholesterol has not been investigated extensively, but there is little variation between races living under widely differing nutritional and climatic conditions. With regard to subjects living in the Netherlands Indies, Radsma reported that 16 Europeans had an average blood cholesterol value of 191 mg., with a range of 130 to 290 mg., per hundred cubic centimeters, 33 native teachers and students 206 mg. and 45 servants and coolies 163 mg., the ranges approximating that of the Europeans. Gross found the blood cholesterol of East Indian natives about 40 mg. per hundred cubic centimeters less than that of Europeans or Americans living in the same region. Stone, after his study of South Rhodesian Negroes, suggested that these differences may be due to dietary factors, having

traced low cholesterol levels in these subjects to a low intake. Negroes living in the United States do not differ from whites in concentration of blood cholesterol. Blood cholesterol levels of Koreans are not significantly different from those of other races (Kim). These scattered data obviously need amplification before conclusions may be drawn as to the effects of heredity and dietary habits on the level of the blood cholesterol. Determinations on Eskimos, who live under dietary and other conditions differing widely from other races, reveal that such factors probably have little influence on the cholesterol levels (Corcoran and Rabinowitch).

Other environmental factors are temperature, pressure and light. Experimental work on dogs (Rabbeno) and rabbits [11] indicates that a decrease in atmospheric pressure causes a rise in free and total cholesterol, but the results of Müller and Talbott on human beings are not confirmatory. They found no change in the cholesterol or other lipids of the blood of 4 healthy young men raised from sea level to an altitude of 14,000 feet. Daily determinations made at the high altitude for thirty-nine days gave constant values. In a recent investigation by McLachlan on dogs and cats, reduction of the atmospheric pressure to less than one-half the normal value led to no change in any of the lipid fractions, whether the animals were in the fasting or the absorptive state. In rabbits the picture differed. After three hours at the low pressure there occurred a substantial decrease in neutral fat, a less pronounced decrease in phospholipid and no change in cholesterol. After six hours at the low pressure the values were at a normal level.

Well controlled experiments on the effect of external temperatures are lacking, but several authors [12] reported that no seasonal variation in the blood cholesterol was found. The effect of increases in the body temperature on the cholesterol level will be discussed later in connection with fevers and infections.

The effect of electromagnetic radiation of various wavelengths has been studied, but the results are contradictory. Malczynski [13] reported that radiation from a quartz lamp produces a temporary increase in blood cholesterol. A similar rise was observed on irradiation with roentgen or infraroentgen, solar and even infra-red rays. Kasatkin and Bugdanova observed this transient increase, but Ornstein reported that the variations in the blood cholesterol of 25 patients treated with ultraviolet rays were not significant. The studies of Harnes (1929) on the effect of various light environments on the blood cholesterol of

11. Griffel. Starup. Wischnowitzer.
12. Boyd, 1938. Man and Gildea, 1937.
13. Malczynski. Malczynski and Lankosz.

rabbits did not establish any significant differences between animals kept in total darkness and those subjected to visible or ultraviolet radiation.

A factor which probably has not received the attention it deserves is the emotional state of the subject at the time of bleeding. Lyons observed that cats within twenty to forty minutes after a period of excitation had hypercholesteremia, the cholesterol being 25 to 30 per cent above the original level. Cholecystectomy did not influence the observed increase, thus ruling out the possibility of a sudden contraction of the gallbladder, followed by resorption of the cholesterol, but the effect was abolished after sympathectomy. Additional evidence for the effect of the mental state on the blood cholesterol has been obtained by Dobreff, Peneff and Wittkower. Of 9 patients from whom blood was drawn immediately before an operation, 6 had a significant increase of blood cholesterol, 1 a slight increase and 2 a slight decrease. Of 16 subjects whose blood was drawn during hypnosis, 11 had a slight to great decrease, whereas 5 had the same or only slightly decreased values.

The effect of muscular work on the blood lipid levels has been investigated by Fahrig and Wacker. After fifteen to eighty minutes of extreme muscular effort increased values were observed for all the lipid fractions, including free and total cholesterol; the data, however, are meager.

CHANGES DURING MENSTRUATION AND PREGNANCY

Exceptions to the usual constancy of the blood cholesterol are the changes in women during menstruation and pregnancy. From data obtained from determinations of the lipid content covering twenty-six monthly cycles in 16 normal young women, Okey and Boyden showed that definite changes in the blood cholesterol levels occurred. The usual fluctuations during the menstrual cycle involved a slight premenstrual rise, followed by a distinct fall immediately before or during menstruation, then a sudden rise during or slightly after the bleeding phase, followed by a gradual decline to the average value. These changes occurred over a period of two weeks and usually were not paralleled by similar fluctuations in the fatty acid and "lecithin" levels. Kaufmann and Erdmann also observed a fall in blood cholesterol during menstruation, which involved principally that in the free form. During the intermenstrual period, about 70 per cent of the total cholesterol was in the form of the ester, whereas during menstruation 85 to 90 per cent of the cholesterol was esterified. These regular variations were considered to be related to ovarian function, as they were abolished by ovariectomy or the physiologic climacteric. In a later publi-

cation Muhlbock and Kaufmann confirmed the intramenstrual fall in the blood cholesterol but reported that the decrease was confined to the esterified cholesterol. Offenkrantz also found a decrease in the total serum cholesterol at the onset of menses, and a rise at the end of the bleeding phase. The changes were mainly in the ester cholesterol fraction and were slight.

That the cholesterol content of the blood increases during pregnancy was pointed out many years ago by Grigaut (1913) and Hermann and Neumann and has been confirmed by many reports which have been summarized adequately by Boyd (1934) in his excellent review on lipemia of pregnancy. From the consensus of studies on this subject, the lipid picture in the blood of pregnant women may be described as follows: During the first trimester there is no change. A gradual rise in all the lipid constituents begins with the fourth month and continues up to the eighth month. The levels thereafter remain high through the puerperium, then decline gradually to normal. Tyler and Underhill observed increases in the levels of free and combined cholesterol and phospholipid, beginning at the third month and continuing to term, after which the levels remained high about two weeks. The ratio of free to total cholesterol and that of phospholipid to cholesterol were fairly constant throughout the period. Boyd (1934) has shown that the lipemia of pregnancy involves changes only in the plasma, the erythrocyte lipids remaining constant. The greatest changes were in the neutral fat fraction, which began to rise in the first trimester and at term was about 100 per cent above the level observed in the absence of pregnancy. The phospholipid and the free and the combined cholesterol began to rise in the second trimester and at term were about 25 per cent higher on the average than in nonpregnant women, though the relative proportions of these substances were unaltered. Similar findings have been reported by Muhlbock and Kaufmann regarding the constancy of the ratio of free to total cholesterol during pregnancy.

The recent publication of Schwartz and associates is of especial interest in that the changes in the blood lipids of the same subjects were followed from the beginning to the end of pregnancy. In general, the changes resembled those in previous studies, namely, moderate increases in free and esterified cholesterol, phospholipid and glyceride, with maintenance of a fairly constant composition of the lipid fraction.

The lipemia of pregnancy, established beyond question in the human species, has not been observed invariably in animals. No variations were noted in the blood lipids of rats (Kaufmann and Erdmann) and dogs (Baumann and Holly, 1926) during pregnancy, and in herbivora there appears to be an actual decrease in the blood lipids during this period. Baumann and Holly (1926) observed in pregnant rabbits

decreases in the cholesterol and the phospholipid to about one-half the values for nonpregnant ones. The decrease occurred during pregnancy even in thyroidectomized rabbits, whereas normally thyroidectomy raises the lipid levels. Boyd (1936 h) confirmed these results and found decreases in this species even in pseudopregnancy. In cows the "dry" period of pregnancy was characterized by low lipid levels in comparison with the period of lactation.[14] Several reports, however, indicate that blood cholesterol may be raised during pregnancy in cows (Sato), rabbits (Kawaguchi) and horses.[15]

Many attempts to explain the lipemia of pregnancy have been reviewed by Boyd (1934), who himself suggested that the increased lipids may act as a "pressure head," forcing the lipids from the maternal blood through the placenta into the fetal circulation. Boyd and Wilson showed that large quantities of lipids pass through the placenta and are removed by the fetus. The amount of lipid absorbed to satisfy the nutritional requirements of the growing fetus was estimated to be of the order of 40 to 50 Gm. per day. The major portion, phospholipid, constituted about 75 per cent of the total. A small, though definite, amount of cholesterol likewise is removed. Although Boyd thus proved that cholesterol and other lipids are supplied to the child by the mother, it is doubtful if the lipemia plays a part, for lipemia does not occur in all species during pregnancy.

Boyd (1935 f) studied the variation in the blood lipids of women during the puerperium. He found that the plasma lipids began to decline almost immediately after the birth of the child, though the decrease was confined almost exclusively to the neutral fat fraction. The phospholipid and cholesterol values remained relatively constant. A significant difference existed between nursing and non-nursing mothers. If normal lactation ensued, the puerperal drop in lipids continued; if the breasts were "dried up," a secondary rise brought the level to or above that of pregnancy.

EFFECT OF DIET ON THE BLOOD CHOLESTEROL

Effect of Hunger.—The effect of fasting was studied first by Gardner and Lander, who observed in cats and rabbits considerable increases in free and esterified cholesterol during periods of hunger. These observations were confirmed by Shope (1927) in human beings, pigs, cats and guinea pigs. During periods of fasting up to six days, hypercholesteremia developed, which decreased a few hours after feeding. The decrease in the blood cholesterol occurred regardless of the type of food fed (fat, carbohydrate, protein or mixed). Similar findings

14. Maynard and others. Shope and Gowen.
15. Brocq-Rousseau and others. Muhlbock.

in rabbits were reported by Fahrig and Wacker. Man and Gildea (1936), on the other hand, reported a study of 10 malnourished patients in whom the cholesterol content varied directly with the state of nutrition. Decreased cholesterol during inanition was reported many years earlier by Terroine, but examination of the protocols shows the changes to have been insignificant.

In regard to rats, Sure, Kik and Church found no changes in the blood cholesterol during fasts up to twenty-six days, though large decreases were observed in the fatty acids and phospholipid. A recent study indicates that animals may be starved to a considerable loss in weight without affecting the levels of the blood lipids. Entenmann, Changus, Gibbs and Chaikoff reported no significant changes in the concentrations of cholesterol, phospholipid and fatty acids in the blood of dogs fasting from four to thirty days or chronically undernourished for several months. In the acute fasting experiments, in which only salts and vitamin concentrates were given, all the lipids were maintained near the control levels up to thirty days. In chronic undernutrition extending up to five months and resulting in a loss in weight of 50 per cent, some lowering of lipid levels was found, being greatest for the fatty acids.

Effect of Diet.—The effect of diet on the concentration of cholesterol in the blood over extended periods was reported first by Luden, who by self observation found that a prolonged vegetable diet resulted in a decreased value for cholesterol and an exclusive meat diet in increased values. Gardner and Gainsborough (1927 c), also on the basis of observations of a single person, reported that the blood cholesterol level varied with the amount of cholesterol in the diet. Turner and Steiner observed 9 patients for twelve to fourteen months and concluded that no relation existed between the type of diet and the blood cholesterol values. Neither maintenance on a high or a low fat diet nor addition of cholesterol to the food influenced the blood cholesterol. Okey and Stewart determined cholesterol in the whole blood of 4 normal women who for approximately one month were kept on diets containing varying amounts of cholesterol. Values were slightly higher when the diet contained 3.1 Gm. of cholesterol per day than when a control diet was used, containing only 0.77 Gm. Values were higher when the cholesterol was administered in the form of egg yolks instead of pure cholesterol mixed with butter. The differences were slight, however. Corwin reported that long term feeding of a high fat diet produced only slight hypercholesteremia in dogs, but when the high fat diet was supplemented with lecithin there was marked elevation of free and combined cholesterol. Cholesterol administered in the solid state without fat had no effect on the blood levels.

The specific effect of lecithin on the level of the blood cholesterol in persons to whom it was fed was investigated by Steiner and Domanski. Ten patients were given 100 Gm. of egg yolk powder daily for six to ten weeks. The daily intake on this diet was 14 Gm. of lecithin and 8 Gm. of cholesterol. The serum cholesterol rose 40 to 218 mg. per hundred cubic centimeters, with an average increase of 101 mg. Similarly, in 4 dogs the same diet resulted in increases in the cholesterol ranging from 155 to 251 mg. per hundred cubic centimeters. Treadwell and Eckstein effectively demonstrated the lack of influence of fat feeding on the blood cholesterol levels in rats. The average values for free and combined serum cholesterol were the same for groups of animals fed diets containing 6 and 28 per cent fat.

Thus, ingested cholesterol, alone or mixed with fat, has little or no influence on the level of cholesterol in the blood, but when mixed with lecithin it produces appreciable rises in the level, probably through finer emulsification and more efficient absorption.

The interesting experiment reported by Tolstoi in which Stefansson, the Arctic explorer, and a friend, two apparently healthy men, subsisted for one year on meat alone is worthy of note. Their diet, in which fat comprised about 75 per cent of the total calories, contained an estimated 2 to 5 Gm. of cholesterol per day. In one the plasma cholesterol rose slightly, from an initial value of 263 to a maximum of 315 mg. per hundred cubic centimeters, in a few days, fluctuated between these values throughout the experiment and at the end was actually lower than at the start, 212 mg. In the other, unfortunately, the initial value was not obtained. During the course of the experiment the values ranged from 268 to 800 mg. per hundred cubic centimeters, the final value being 415 mg. After four weeks on a general (low cholesterol) diet, the value decreased to 200 mg. per hundred cubic centimeters. The results do not disclose any clearcut effect of a long term, high cholesterol diet on the plasma levels of this lipid, but they emphasize the factor of individual tolerance.

Reports regarding diurnal variations in the blood cholesterol disagree, but the weight of evidence indicates that the changes are not significant. Iscovesco claimed to have observed two to three maxima in the blood cholesterol throughout the course of a day, corresponding to periods six to eight hours after meals, but this has not been borne out by later investigations. Variations found during the course of a day by McEachern and Gilmour, and Fröhling could not be correlated with the type or the amount of food fed. Munoz, and Bruger and Somach reported the diurnal cholesterol level to be unaffected by the type of diet and found only slight variations. In determinations carried out every two hours for twenty-four hours on 9 subjects

(Bruger and Somach) the variations in the whole blood cholesterol averaged less than 8 per cent. Over a four hour period, either absorptive or fasting, the standard deviation was only 4 per cent. Results were similar in a careful study carried out by Boyd (1935 c). Eight adult subjects on a balanced diet of three meals per day were studied. Blood was drawn at intervals during the day and night. The cholesterol and other lipid values were not affected by such factors as time of day, intake of meals or sleep. The level remained quite constant. Daily variations expressed as standard deviations from the mean were 6.5 and 7.2 per cent for free and ester cholesterol, respectively—one-half to one-third the variation among different persons.

Alimentary Hypercholesteremia. — Early observations [16] indicated that an increase in the blood cholesterol occurs after ingestion of food which may contain little or no cholesterol. McClure and Huntsinger reported that the cholesterol levels rose after adults were fed meals of oleic acid or olive oil. Page, Pasternak and Burt, and Wendt, found increases in the blood cholesterol occurring within four hours when 100 Gm. of olive oil was fed to normal fasting subjects. Others have reported that ingestion of large quantities of fat had no influence on the blood cholesterol in normal and nephritic subjects (Hiller and associates), normal and xanthomatous patients (Chaikoff and co-workers), children (Wilson and Hanner) and dogs.[17] Blotner attempted to apply the changes in the cholesterol content of the blood occurring after ingestion of fat as a diagnostic criterion in various clinical conditions. The method is similar in principle to the sugar tolerance test. Blood is taken before, and at hourly intervals after, ingestion of 1 pint (453 cc.) of 20 per cent cream, equivalent to about 100 Gm. of fat. Normal persons show no significant changes in an eight hour period. In a group of thin persons the blood cholesterol remained constant or decreased slightly; in a group of 21 obese but otherwise apparently normal subjects the levels during fasting were higher than in a similar group of normal controls, and the cholesterol curve was characterized by a considerable increase at six hours, followed by a gradual decline. These results in obese persons contradict data reported by Rony and Lævy, who found no definite change in the plasma cholesterol values of 18 markedly obese subjects after these had ingested 1 pint of 20 per cent cream.

The high cholesterol values sometimes found in cases of advanced or uncontrolled diabetes have stimulated investigation of the effect of dextrose on the cholesterol level. Remesow and Mattrosowitsch reported that cholesterol and dextrose varied inversely in the blood. Partly on this basis, they concluded that cholesterol may be converted

16. Autentrieth and Funk. Lifschutz. Lindemann.
17. Bloor, 1933. Rubin.

to carbohydrate. Mosenthal studied the effect of the ingestion of dextrose on the level of dextrose and that of cholesterol in the blood. He found that the rise in blood sugar was accompanied by wide variations in the cholesterol concentration, but no regular behavior was observed; the cholesterol increased, decreased or remained constant. The author concluded that osmotic phenomena may have played a part in the variations observed. Fitz and Bruger, in carrying out the usual dextrose tolerance test on 20 patients, observed an increase in the cholesterol ester fraction of the blood serum of 12. In the remaining 8, it was constant or decreased slightly. Sperry (1937 h), on the other hand, was unable to confirm these results, and concluded that no significant variation in the cholesterol occurred as a result of the ingestion of dextrose. The experimental work of Fitz and Bruger was criticized on the basis that their subjects were bedridden patients, many of whom exhibited cachexia and conditions such as infectious arthritis, carcinoma and hepatic cirrhosis.

Bruger and Poindexter reported that intake of large quantities of water had no effect on the level of the blood cholesterol; with water accompanied by varying quantities of urea, slight lowering of the cholesterol levels was observed, which probably was not significant.

Hypercholesteremia Following Ingestion of Cholesterol.—The notion that the blood cholesterol is increased after the ingestion of this lipid rests on the results of Bürger and Habs, who substantiated many similar earlier observations. They studied a group of fasting normal persons, who were given 100 cc. of olive oil containing 5 Gm. of cholesterol or cholesterol esters. In each instance lipemia occurred within four hours, accompanied by considerable increase in the free and the combined cholesterol. The levels declined thereafter and reached the normal fasting value within twelve hours. The negative results of previous investigators in similar experiments [18] were attributed to the administration of insufficient cholesterol, the use of inadequate analytic methods or the use of too little fat in admixture with the cholesterol. Gardner and Gainsborough (1927 c) were unable to confirm this and concluded that there is no relation between the amount of cholesterol ingested and the level of this lipid in the plasma. They criticized the results of Bürger and Habs because an inadequate method of extraction had been used. Bürger (1928) applied this procedure as a diagnostic test in a variety of conditions. Barreda, however, carried out the same procedure in normal and in abnormal subjects without observing the consistent changes reported by Bürger. Inasmuch as large quantities of cholesterol must be ingested to produce even questionable hypercholesteremia, this condition apparently does not occur when cholesterol is taken in physio-

18. Campbell. Cohn and Heimann. Mjassnikow, 1926. Rothschild, 1915. Ssokoloff.

logic quantities, and under normal dietary conditions the fluctuations in the concentration of cholesterol in the blood appear to be of little importance.

Effect of Cholesterol Feeding in Herbivora.—Though in man and in other omnivora dietary cholesterol has little or no influence on the level of this lipid in the blood, in rabbits the reaction to the ingestion of this substance is marked. When cholesterol is added to the food of these animals to the extent of only a fraction of a per cent of the diet, the blood levels are raised and cholesterol is deposited extensively throughout the body. Since the deposit of lipids in the large arteries of rabbits fed cholesterol resembles human atherosclerosis, a voluminous literature has accumulated which has been reviewed by Schoenheimer, (1924) Duff and Anitschkow. The pioneering work of Wacker and Hueck established that the blood cholesterol of rabbits, normally low, hav-

Results Obtained in Two Investigations of the Effect of Cholesterol Feeding in Rabbits

Authors	Free Cholesterol, Mg. per 100 Cc.	Total Cholesterol, Mg. per 100 Cc.	Phospholipids, Mg. per 100 Cc.	Glycerides, Mg. per 100 Cc.	Total, Mg. per 100 Cc.
Page and Bernhard*...	356	1,435	526	270**	2,902
Weinhouse and Hirsch†	452	1,652	706	718	3,060

* Cholesterol, 0.2 Gm. per day, was administered as a solution in oil. The determinations were made on heparinized plasma.

† Cholesterol, 1.0 Gm. per day, was administered without added fat. The determinations were made on serum.

** This value was calculated from the authors' figures by subtracting the several lipid fractions from the total lipid.

ing average values of 20 and 50 mg. per hundred cubic centimeters for free and total cholesterol, respectively, increased after several months of cholesterol feeding to approximately twenty times these values. Versé confirmed these results but observed that the high cholesterol levels are reached much more quickly and with smaller daily feedings of cholesterol when it is administered as a solution in oil by means of a stomach tube. Cholesterol unquestionably is absorbed and rises to high levels in the blood without the addition of oil.[19] The only complete determinations of lipids in cholesterol-fed rabbits were carried out by Page and Bernhard and Weinhouse and Hirsch. The effect of added fat is well illustrated by the fact that in the two studies approximately equal values for free and total cholesterol were found though in the latter investigation five times as much cholesterol was administered as in the former, in which oil was used as a vehicle. The accompanying table

19. Bruger and Fitz. Meeker and others. Turner and Bidwell. Weinhouse and Hirsch.

shows the values reported for the separate lipid fractions at the height of the hypercholesteremia.

In spite of the difference in the administration of the cholesterol in the two studies, approximately the same relationships are noted between the free and the combined cholesterol, and phospholipids, though the low value for glycerides in the animals on the high fat diet is puzzling.

Weinhouse and Hirsch observed a gradual decline in the values for all the blood lipids after the maximum levels had been reached in about three months. They attributed this to impairment of the absorption of food as a result of infiltration of lipids into the transporting cells of the small intestine, which was observed histologically. The gradual loss in weight which paralleled the decline in lipid values substantiates this hypothesis.

No satisfactory reason has been found as to why rabbits differ from omnivora in their ability to dispose of dietary cholesterol. Schoenheimer pointed out that rabbits and guinea pigs differ from omnivora in that they do not have alimentary lipemia [20] after a single large feeding of fat. They are the only animals in which lipoidosis occurs as a result of cholesterol feeding. It seems reasonable to assume that the rabbit is reacting to a substance foreign to its diet by virtue of its herbivorous nature. But all animals, including rabbits, contain cholesterol as a normal component of their tissues and body fluids, and presumably possess mechanisms for its excretion, destruction and synthesis. It is difficult to understand, therefore, how the rabbit can distinguish between cholesterol of exogenous and cholesterol of endogenous origin. It undoubtedly lacks some excretory or metabolic function, possessed by omnivora, for the removal of exogenous cholesterol. At present, no anatomic or physiologic difference is known to account for this unusual behavior. With the exception of guinea pigs,[21] which react similarly to rabbits, there are no data on whether or not the susceptibility to lipoidosis following cholesterol feeding is shared by other herbivora.

PHYSICAL STATE OF CHOLESTEROL IN THE BLOOD

Under ordinary circumstances the blood plasma is clear and transparent in spite of the large amounts of cholesterol, glycerides and other lipids in it. As these substances are extremely insoluble in aqueous mediums, it is assumed that they are present in the blood in colloidal form. Studies of the colloidal properties of aqueous solutions of cholesterol and its esters, alone or combined with other lipids, have not been of great value in elucidating the state of these substances in the blood. The physical properties of cholesterol sols reported by various

20. The term "lipemia" denotes the typical milky appearance of the serum determined by visual observation and has no chemical significance.

21. Rothschild, 1915. Wacker and Hueck.

investigators should be viewed with caution because in most instances the materials used were highly impure and because the properties in question often are affected profoundly by the method of preparation of the sols. Moyer, for example, observed considerable divergence in the isoelectric point and electrophoretic mobility at p_H 5.8 of cholesterol sols prepared according to the directions of other investigators.[22]

In general, colloidal solutions of cholesterol and its esters are difficult to prepare, are precipitated easily by low concentrations of salts, even when stabilized by phospholipids,[23] and always have a milky appearance even in great dilution. According to Remesow (1936), sols of highly purified cholesterol cannot be prepared, for they coagulate at the instant of formation even with the lipid in extremely low concentration.

The relative stability of the plasma lipids as compared with their colloidal solutions in vitro suggests a combination with the plasma proteins; indeed, most investigators agree that the lipids are "peptized" in this manner. As evidence of such a combination it has been repeatedly demonstrated that only a fraction of the lipids can be extracted from plasma by the usual lipid solvents and that they tend to remain with the protein fractions precipitated by salts (Turner and Gibson).

When serum is treated with solvents, such as ether or chloroform, the lipids are removed only incompletely.[24] The amount that can be extracted varies with the p_H, being greatest at 5.5 to 6.0, the isoelectric point of globulin and fibrinogen.[25] Over the p_H range of 1.7 to 13.3, only a small fraction of the total is extracted with ether (Delage, 1936 a). If a small quantity of lower alcohol or acetone is added to the serum, the greater part of the lipid is extractable by ether (Delage, 1936 b). This is attributed to a decrease of the interfacial tension between the serum and the ether, resulting in more intimate contact between the latter and the protein-lipid micelles; but it may likewise be attributed to denaturation of the protein.

The precipitation of cholesterol with the salting out of proteins has been observed by many. The early literature was reviewed by Turner and Gibson. The amounts of the various lipids precipitated with the protein fractions differ according to variations in the experimental procedures employed, with differences in findings between many investigators; but all agree that relatively large proportions are combined with the protein of the serum.

Turner and Gibson fractionated the proteins of horse serum and human and dog plasma by salt precipitation and determined fatty acids, phospholipids and cholesterol in these fractions. About half the total

22. Eagle. Kermack and MacCallum. Keeser. Porges and Neubauer. Rona and Deutsch. Stern.

23. Remesow, 1936. Stern.

24. Achard. Delage, 1936 a. Handovsky and others. Neuschloss. Terroine.

25. Delage, 1936 a. Theorell.

lipids were carried down with the proteins. Greater amounts were associated with the globulin than with the albumin. For all species, about 70 per cent of the free and of the total cholesterol was carried down with the proteins and was equally distributed between the albumin and the globulin.

By successive fractionation of horse serum with ammonium sulfate at p_H 3.8, Macheboeuf isolated an albumin fraction containing 22 per cent "lecithin," 18 per cent cholesterol esters and 60 per cent protein. In spite of its high lipid content, this substance was very soluble in water. Clear solutions containing more than 50 Gm. of lipids per liter and clear gels containing more than 100 Gm. per liter were obtained. The lipids could not be extracted directly from these solutions by ether but were extractable after the protein had been coagulated with boiling alcohol. The isolated lipids were not soluble in water.

Kleczkowski showed that the amount of lipid as well as carbohydrate combined with the serum albumin depends on the method of the latter's preparation. Crystalline serum albumin of the horse, for example, contained only traces of cholesterol, phospholipid and sugar, whereas the albumin of high lipid content prepared according to Macheboeuf contained as much as 1 per cent of sugar. Albumins prepared in other ways contained varying quantities of lipids and carbohydrate.

Ultrafiltration studies of serum and other body fluids also indicate that proteins are combined with cholesterol. Went and Goreczky showed that the cholesterol concentration in serum ultrafiltrates was proportional to the protein concentration. When the ultrafiltrate was protein free, it was also cholesterol free. The phospholipid, on the other hand, seemed associated predominantly with the euglobulin fraction, since only a small proportion passed through with the protein of low molecular weight.

In similar experiments, Bendien and Snapper reported that neither lecithin nor cholesterol was combined with the albumin but may have been partially associated with the euglobulin. In a study of pathologic pleural and ascitic fluids, Bruger found that though the cholesterol did not pass an ultrafilter permeable to protein it was readily adsorbed on kieselguhr. Bruger expressed the belief that this indicates that cholesterol is not combined with protein, but this argument is not valid. It is probable that the cholesterol, united by loose chemical forces to the protein, has an affinity for kieselguhr great enough to overcome the hold of the protein.

Application to this problem of the improved electrophoresis apparatus recently developed by Tiselius has furnished the most convincing evidence for the presence, in normal serum, of combinations between the proteins and cholesterol. Tiselius showed that serum contained four well defined protein components which migrated at different velocities

in the electrophoresis apparatus; albumin and three globulins, denoted α, β and λ, respectively. By analyzing these fractions, Blix, Tiselius and Svensson demonstrated that all contained cholesterol, with far greater quantities in the α and β globulins than in the other two components of the serum. These fractions were richer also in phospholipid and carbohydrate. Mellander had shown previously by electrophoresis that the albumin and globulin of serum contained cholesterol; also, that a negatively charged cholesterol fraction migrated with a greater velocity than the proteins. Thus, at least a part of the cholesterol is unbound. He also noted cholesterol-free albumin and globulin fractions.

Though protein-lipid combination undoubtedly occurs, the character of the forces involved is not clear. It is doubtful that the lipids are attached as a prosthetic group by the ordinary chemical bonds, for they are removed by comparatively mild procedures not involving the integrity of the protein except possibly for denaturation. Two other possibilities suggest themselves. Combination may occur through the mutual attraction of oppositely charged polar groups, resulting in a uniform distribution of discrete lipid molecules throughout the protein micelle. Or, combination may take place between the protein micelles and high molecular aggregates of lipid molecules, held together by opposite electrical charges or by physical enmeshing as suggested by Bruger (1935 a).

The significance of these protein-cholesterol combinations to the organism other than as a possible means of holding the cholesterol in solution is not established. Wide variations may occur in either component without affecting the other. For example, Schwarz and Lichtenberg found that the hypercholesteremia induced by bleeding had no effect on the serum protein levels, and Page, Farr and Weech observed no consistent changes in the total concentration or the relative proportions of the serum lipids in dogs fed a low protein diet until the serum albumin dropped below 1.5 Gm. per hundred cubic centimeters and gross edema occurred.

Evidence indicates that in contrast to its relative nonreactivity in the molecular state, colloidally dispersed cholesterol is markedly chemically active. For example, Remesow and Mattrosowitsch found that cholesterol sols have a strong reducing action toward the usual sugar reagents. Colloidally dispersed cholesterol and its esters in the presence of air or of hydrogen peroxide can dehydrogenate polyamines and phenols (Remesow and Sepalowa, 1933, 1935) and under certain conditions may have a catalase activity (Remesow, 1934). These findings suggest the possibility that cholesterol may take part in cellular respiration. Further work along these lines is obviously necessary.

THE CHOLESTEROL OF THE RED CELLS

The reported values for the concentrations of cholesterol in the red blood corpuscles of normal human beings range from 62 to 240 mg. per hundred grams of cells. Grigaut and L'Huiller reported values from 130 to 177 mg.; Bloor (1916 c), 170 to 240 mg.; Oser and Karr, 62 to 156 mg.; Laroche and Grigaut, 150 to 165 mg.; Brun, 127 to 149 mg., and Boyd (1936 g), 150 ± 19 mg. These data show that though the average values differ considerably, probably reflecting differences in analytic procedures, the range of variation reported by each author is narrow compared with the normal range of variation observed in the serum.

In contrast with that of the serum, the cholesterol of the erythrocytes is mainly, if not entirely, in the free form, though investigators disagree as to the actual amount of combined cholesterol present. Richter-Quittner, Iwatsuro, Brun, Sperry (1935) and Rubin were unable to find statistically significant quantities of combined cholesterol in the cells of human beings, but others [26] reported slight, though definite, amounts. In animals as well, some investigators [27] found minimal quantities of ester cholesterol or none, whereas others observed appreciable amounts.[28] How much of this disagreement is due to errors in the analytic methods is impossible to estimate at present. Aside from the usual sources of error encountered in determinations of cholesterol, one applies particularly to the red cells. When the cellular concentration of a substance is calculated indirectly from the difference between whole blood and plasma values on the basis of the hematocrit reading, the error is large, because the concentration must be calculated from three experimentally determined values, each of which is subject to some error. This criticism applies especially to combined cholesterol, the concentration of which in the cells is low and which itself is not determined directly but is calculated from the difference between the total and the free cholesterol. Consequently, the direct method, in which a measured portion of the cells is analyzed after preliminary separation of the plasma, is preferred. After comparing the direct and the indirect method for the determination of corpuscular lipids, Boyd (1936 g) concluded that only the former yields reliable figures.

The cholesterol content of the erythrocytes undoubtedly remains constant under conditions in which there is considerable variation in that of the plasma. Boyd (1934 e) has shown that the cellular lipids are unchanged in the chronic lipemia of pregnancy, of diabetes and of hemorrhage. These observations were confirmed strikingly by Rubin,

26. Boyd, 1936 g. Cytronberg. Erickson and others. Rosenthal.
27. Bodansky, 1925. Knudson. Wacker and Hueck.
28. Barreda. Pfeiffer. Rohmann.

who found normal values for cholesterol and the other lipids of the red cells in a diabetic patient whose plasma showed 1,600 mg. of cholesterol and 23,000 mg. of total lipids per hundred cubic centimeters. In alimentary as well as in chronic lipemia no change is noted in the cholesterol of the red cells. Bloor (1916 b) and Rubin with dogs, and Iwatsuro, with rabbits, observed no increase in the concentration of cholesterol in the red corpuscles after feedings of fat with or without cholesterol. Similar observations were made by Wendt, Fröhling and Boyd and Twedell in human subjects. Slight increases in the cellular cholesterol after cholesterol feeding were reported by Knudson and Bodansky (1931) in dogs, and by Henes, Richter-Quittner and Brun in human beings. Brun found no variations in the cholesterol of the red cells in human beings over periods of one day or a month. He observed no effect of age.

Though the variations in plasma cholesterol are not reflected in the erythrocytes, there are some indications that the latter undergo changes in their cholesterol concentration under certain conditions. Boyd (1936 g) observed increases in the red cell lipids not related to similar changes in the plasma lipids in cases of infection and of anesthesia induced with ether. Bloor (1916 c) observed increased cholesterol in the red cells of only a few of a large group of subjects suffering from various pathologic conditions; in the majority the cholesterol content was within normal limits. Brun reported slightly higher than normal values for patients suffering from cancer and markedly higher values for those suffering from jaundice, the values decreasing as the conditions improved. The increases in the jaundiced patients were attributed to the enhanced absorption of cholesterol under the influence of the bile acids, which were at high levels in the blood in this condition.

Bugnard observed that the venous plasma of dogs contained more cholesterol than the arterial plasma, whereas the concentration in the whole blood was unchanged. He concluded that there was an exchange of cholesterol between the corpuscles and the plasma depending on the p_H of the circulating blood. According to his theory, when carbon dioxide is given up by the blood in the lungs, and the blood becomes relatively alkaline, cholesterol migrates from the plasma to the corpuscles. Then, as the blood again is saturated with carbon dioxide during its passage through the tissues, the reverse change takes place. In confirmation, Bugnard found in experiments in vitro that saturation of whole arterial blood with oxygen gave a plasma poorer in cholesterol than when the blood was saturated with carbon dioxide. Similarly, acidification of whole blood with ammonium acid phosphate produced a distinct rise in the plasma cholesterol. Levine and Soskin also found an inverse relation between the total serum "fat" and its carbon dioxide–combining power. The "fat" content of the whole blood remaining

unchanged, they postulated a labile exchange of "fat" between the corpuscles and the serum under the influence of the acid-base balance.

If such a process occurred under the influence of the p_H, the oxygenated serum leaving the lungs should contain less cholesterol than the venous blood serum of the right side of the heart. Shillito, Bidwell and Turner determined the cholesterol content of whole blood and serum taken simultaneously from the superior vena cava and the carotid artery. For a given animal (dogs and cats were used) the levels were the same. Thus no effect of passage through the lungs on the relative proportions of cholesterol in the red cells and the serum could be demonstrated.

Studies have shown that the lipids of the erythrocyte are not distributed uniformly throughout the cell, but are associated with the stroma.[29] Indeed, they are bound so firmly as to withstand repeated washing with buffer solution. Analyses of stroma from several species of animals show varying absolute quantities of lipids, but the relative proportions remain fairly constant at about 60 per cent phospholipid, 30 per cent free cholesterol and 10 per cent cholesterol esters and neutral fat (Erickson and co-workers). Essentially similar values have been reported for the erythrocytic lipids of a large variety of mammals and fish (Dzemian). The amounts of cholesterol and other lipids appear related to the size of the cell; the larger the cell, the greater the percentages.

The chemical and physical properties of the erythrocyte suggest that it is surrounded by a gel-like membrane or envelope several molecules thick, consisting of a complex of lipid and protein.[30] Thus it is possible that cholesterol in combination with other lipids and the stromal proteins plays some part in determining the properties of the intact cell, though what properties are affected is not known. No relation was found between the cholesterol content of a cell and its osmotic resistance (Williams and co-workers) nor between the lipid content and the permeability to lipid-soluble substances (Dzemian). The fact that the cholesterol concentration is constant and the fact that the lipid is probably entirely in the free form, is independent of fluctuations in the plasma cholesterol and is associated with the stroma indicate it has no metabolic function in the red cell but has a structural role in combination with proteins and the other lipids.

The other cellular component of the blood, the leukocytes, contains much more lipid than the red cells. Boyd (1933 b), in a study of the white blood cell lipids of 8 normal young women, found that the con-

29. Brun. Erickson and others.

30. Dzemian. Erickson and others.

centration of total lipid in these cells varied between 1 and 3 per cent, about four times their concentration in the plasma. The lipid fraction is composed of 47 per cent phospholipid, 31 per cent neutral fat and 11 per cent each of cholesterol and cholesterol esters. In general, the range of variation of these fractions was considerable. The white cells resemble body tissues, such as the heart, liver and brain, in their high total lipid and a relatively large proportion of phospholipid but resemble the plasma in their high content of cholesterol esters.

SUMMARY

The concentration of cholesterol in the serum of normal human beings of both sexes averages about 200 mg., with a range of from approximately 100 to almost 400 mg., per hundred cubic centimeters. Physicochemical studies of the blood serum indicate that the cholesterol is at least partially combined with the serum proteins and possibly other lipids in a colloidal complex, the structure and the composition of which are as yet unknown. In spite of the wide variance in the total cholesterol, the proportion of the free in the total cholesterol fluctuates within narrow limits, averaging about 30 per cent. This ratio is probably maintained through the continuous and simultaneous esterification of free cholesterol and hydrolysis of the esters.

Except for a pronounced rise during the first few days of life and a slight rise during childhood, the cholesterol level remains constant. A steady increase in the cholesterol with age in the absence of any metabolic abnormality has been reported by some investigators, but this has been disputed by others. The evidence indicates that during adult life the cholesterol of an individual tends to maintain a constant value uninfluenced by environmental conditions or living habits.

Though many contradictory reports have been published regarding the effect of diet on the serum cholesterol, certain conclusions may be drawn. Long periods of fasting produce no significant changes in the blood levels. Similarly, the character and the quantity of food ingested, whether taken over long or short periods, have little effect on the concentration of cholesterol. Though several authors have observed alimentary hypercholesteremia, particularly after the feeding of meals high in fat, the increases were not striking. Indeed, many investigators have been unable to detect hypercholesteremia even after the feeding of meals rich in cholesterol. It is noteworthy that when significant elevations of the cholesterol level occurred after the ingestion of this substance, the amounts fed were so large as to be unphysiologic. Apparently, in man hypercholesteremia normally does not develop as a result of the ingestion of moderate quantities of cholesterol.

II. CHOLESTEROL METABOLISM

The previous section disclosed that the concentration of cholesterol in the blood of any person remains at a constant level, not easily changed by a diversity of physiologic conditions. In the body, cholesterol is constantly or intermittently synthesized and destroyed, absorbed and excreted, mobilized from and deposited in the tissues. The level therefore represents a dynamic balance between synthesis, absorption and mobilization, which tend to add cholesterol to the blood stream, on the one hand, and destruction, excretion and deposition, which tend to remove it, on the other. Any change in the level of the blood cholesterol, therefore, is a result of a disturbance in one or more of these processes. The proportion of free to esterified cholesterol also represents a dynamic equilibrium between continuous esterification and saponification in the blood or the tissues. Present knowledge of these interrelated and probably interdependent processes will be reviewed briefly.

SYNTHESIS

Because of the polyterpenoid nature of the cholesterol molecule and the ubiquitous occurrence of terpene structures in plants, in contrast to their rarity in the animal kingdom, early chemists were reluctant to admit that animals were able to synthesize these compounds, and (on the mistaken notion that cholesterol and plant sterols were isomeric) believed that cholesterol was entirely exogenous in origin. These early views were expressed by Gardner and his collaborators (Ellis and Gardner, 1909).

The first conclusive evidence for the synthesis of cholesterol was reported in 1920 by Gamble and Blackfan. In balance experiments on infants they found a greater amount of sterol excreted than was ingested. Since then, this concept has been verified by a variety of studies on man and animals.[31] Even in pregnant women, in whom there is a large requirement of sterol for the growing fetus, a negative sterol balance was observed (Kaufmann and Muhlbock, 1933). Schoenheimer and Breusch, in experiments on mice, found the amount of sterol synthesized to be dependent on the quantity available in the food. With ingestion of large amounts of cholesterol, destruction, not synthesis, occurred.

Herbivora, which have no exogenous supply of cholesterol, must synthesize as much as the body needs. "Schoenheimer and co-workers (Schoenheimer, 1931) by systematic investigations showed that the sterols of plants cannot serve as a source of cholesterol, for they are not absorbed from the animal intestine. Even if phytosterols were absorbed in sufficient amount, their different carbon skeleton would render their transformation into cholesterol extremely unlikely.

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31. Beumer and Lehmann. Channon. Gardner and Fox, 1921. Heinlein. Kaufmann and Muhlbock, 1933. Randles and Knudson, 1925. Schoenheimer, 1929 d

Relatively little is known of the mechanism or the site of the synthesis of cholesterol. Such lower plants as the yeasts and the molds produce ergosterol when the only sources of carbon are sugars, fatty acids or sodium acetate (Bills). Minovici mentioned oleic acid as the precursor of cholesterol in the animal body; the evidence, however, is not convincing. Eckstein and Treadwell [32] suggested that fatty acids play a part in the synthesis of cholesterol. They observed that rats synthesized more cholesterol (as determined by the difference between dietary and fecal sterols) on a high fat than on a low fat diet. The excess was not due to depletion of the cholesterol of the body. There was, moreover, greater synthesis with fats of high than with fats of low iodine number. Discherl and Traut, however, failed to observe an increase in the synthesis of sterols in mice fed esters of stearic, oleic and linoleic acids over a control group fed a basic diet of oats.

Perhaps the best evidence against the conversion of fatty acid to cholesterol is found in experiments employing deuterium (heavy hydrogen) as a tracer. Schoenheimer and Rittenberg [33] administered small quantities of deuterium-containing water to mice until the body water contained 1.5 per cent; this concentration was maintained for varying periods. The concentration of deuterium in the body cholesterol slowly rose to one-half the concentration of that substance in the body water and remained constant. This important fact indicates that in the formation of cholesterol at least half of the hydrogens are exchangeable, a circumstance which could arise only by the coupling of a number of small molecules. The recent results of Sperry, Waelsch and Stoyanoff point in the same direction. In rats fed deuterium-containing fatty acids, there was no measurable uptake of deuterium in the tissue sterols.

On purely chemical grounds the direct conversion of fatty acids to cholesterol is unlikely. The polyterpenoid structure of cholesterol is so different fundamentally from the unbranched carbon skeleton of the fatty acids that any conversion of the latter to the former would be impossible without a complete breakdown of the fatty acid molecule.

The close structural similarity between cholesterol and other substances of biologic origin, such as squalene and vitamin A, has led to some interesting theories regarding the possible role of these substances as precursors,[34] but there is little conclusive evidence pro or con. The weight of evidence favors a mechanism in which cholesterol is built up of small units rather than by direct rearrangement of large molecules.

Though almost every organ has been suggested as the site of the synthesis of cholesterol in the body, little is known in this regard. The

32. Eckstein and Treadwell, 1935, 1938. Treadwell and Eckstein, 1939.

33. Rittenberg and Schoenheimer. Schoenheimer, Rittenberg and Graff.

34. Bryant. Vanghelovici.

liver and the adrenal glands, because of their high cholesterol content, have been most frequently considered. The theory that cholesterol is synthesized in the adrenal glands was cited by Grigaut [35] on the basis of chemical and histologic studies of disease of the adrenal glands and especially in view of the previous observation [36] that hypercholesteremia develops in dogs and rabbits after unilateral adrenalectomy. He regarded the hypercholesteremia as a result of compensatory hyperactivity of the remaining gland. The studies of Baumann and Holly (1923) on rabbits and of Randles and Knudson (1928) on rats effectively refuted this postulate, as neither the former nor the latter investigators observed any changes in the blood cholesterol following unilateral or bilateral adrenalectomy.

The remarkable increase in cholesterol ester content of the adrenal glands of rabbits on a high cholesterol diet [37] suggests that these glands may function as a depot. At any rate, the cholesterol content of the adrenals appears to reflect the changes in the blood. Ewert (1935) has shown that in aseptic or infectious hyperthermia the decrease in the blood cholesterol is paralleled by corresponding changes in the adrenals. The old suggestion of Aschoff that the adrenals play a part in the intermediary metabolism of cholesterol has added significance since the discovery of the steroid nature of the cortical hormones.

The position of the liver as the central laboratory of the body has led to its consideration as the site of the synthesis of cholesterol. Artom and Minovici claimed to have demonstrated synthesis of cholesterol in the liver during autolysis and perfusion, but recent autolytic experiments on liver by Sperry and Brand have not confirmed these results. Experiments of Sperry, Waelsch and Stoyanoff, in which deuterium-containing water was fed to rats, indicate that the liver is no more important for the synthesis of cholesterol than other tissues, for the amount of deuterium in the sterol fraction of the liver was no greater than in other parts of the body. The recent report of Winter suggests that the liver may play a part in the synthesis of cholesterol. In balance experiments on rats whose livers were damaged by carbon tetrachloride poisoning, the sterol "loss" was greater in the experimental than in the control group with normal livers.

Other organs, particularly the brain, the lungs and the spleen, have been considered as sites for the synthesis of cholesterol, but the evidence thereof is not convincing. At present Bills's statement must be accepted: "When all the facts are considered, it appears as likely as not that animal sterols, insofar as they are endogenous, originate in the cells in which they occur."

35. Chauffard and others. Grigaut, 1913.

36. Grigaut, 1913. Rothschild, 1914.

37. Kay and Whitehead. Weinhouse and Hirsch.

DESTRUCTION

It is generally agreed that cholesterol may be destroyed in an appreciable amount in the animal body, and the amount thus metabolized is a function of the quantity in the diet. Dam (1931) studying chicks, Menschick and Page cats and Page and Menschick (1932) and Cook (1937) rabbits observed negative sterol balances when cholesterol was fed. Breusch, extending the previous experiments of Schoenheimer and Breusch, found that whereas on a low cholesterol diet there was a synthesis of about 13 mg. per hundred grams of mouse per day, on a high cholesterol diet there was destruction of about 4.3 mg. These figures represent only the net loss or gain in cholesterol, i. e., the difference between synthesis and destruction. Presumably, the absolute amounts synthesized and destroyed are much higher than the balance figures indicate. Beumer and Fasold attributed the destruction of sterols on high cholesterol diets to bacterial decomposition in the intestine; the weight of evidence, however, is against such a hypothesis. Neither Bischoff nor Dam (1934) observed any destruction of sterols by incubation of feces or of intestinal contents. The fact, demonstrated by Breusch, that only absorbable sterol, i. e., cholesterol, had any influence on the sterol balance is a strong indication that destruction occurs in the tissues and not in the intestines.

For a long time no information was available regarding the breakdown products of cholesterol, but recent data furnish glimpses of the possible route of the metabolism of this lipid. Page and Menschick (1930 b), supported by Schoenheimer (1932), detected spectroscopically a substance characteristic of cholestenone in atherosclerotic arteries, where masses of cholesterol had remained for long periods. Haslewood isolated 7-hydroxycholesterol from ox liver, and the same or an isomeric substance was found in pregnant mares' serum by Wintersteimer and Bergstrom. Apparently the 7 position in cholesterol, which is susceptible to attack in vitro, is also active physiologically. Waelsch and Sperry separated the unsaponifiable matter of deuterium-fed rats and found the ketone and hydrocarbon fractions, as well as the cholesterol, high in deuterium, suggesting a close metabolic relation.

The multitude of sex and adrenal hormones with similar steroid ring systems suggests a common precursor which may well be cholesterol, though no direct physiologic relation has as yet been established. Similarly, cholesterol may be a precursor of the bile acids, though again there is no conclusive evidence for or against this theory.

ABSORPTION

The outstanding characteristic of the absorption of sterols in the animal body is its remarkable specificity. In a brilliant series of papers, Schoenheimer (1931) and his co-workers showed that whereas choles-

terol is absorbed readily in all species the sterols of plant and various derivatives of cholesterol are not absorbed to any appreciable extent. On measuring the extent of absorption by analysis of liver, lymph and feces it was found that the plant sterols (stigmasterol, brassicasterol and sitosterol), the wool sterols (lanosterol and agnosterol) and the four saturated cholesterol derivatives (dihydrocholesterol, coprosterol and their epimers) were not absorbed. Allocholesterol, which differs from the parent substance only in the position of the double bond, was absorbed to a slight extent; this was attributed to the ready isomerization of this substance to cholesterol. Dam and Brun found that dihydrocholesterol, readily absorbed, after two weeks constituted 11 to 13 per cent of the body sterols. Page and Menschick (1930 c) showed that ergosterol may be absorbed to a small extent.

As is true of the other lipids, the mechanism of the transport of cholesterol across the intestinal wall is obscure. It has been demonstrated repeatedly that fats aid in the absorption,[38] but how is not known. The presence of bile also aids in the absorption of cholesterol. Loeffler, extending the observations of Schoenheimer, his conclusions being fully substantiated by Hummel, found that the fivefold increase occurring in the hepatic cholesterol of mice fed cholesterol rose to sevenfold and thirteenfold respectively when the cholesterol was supplemented with desoxycholic and cholic acid. These studies suggest that bile salts aid in the absorption of cholesterol through formation of choleic acids in the manner proposed by Verzár [39] for the fatty acids. It is noteworthy, however, that the greatest effects were obtained [40] with glycocholic and cholic acids, neither of which is able to form choleic acid complexes with cholesterol (Wieland and Sorge).

The question of whether cholesterol is absorbed in the free or the esterified form is still open, though evidence favors the latter. Frölicher and Süllmann, in analyses of thoracic lymph from rabbits, found a large increase in its cholesterol ester content and concluded that esterification had occurred during absorption. Brockett, Spiers and Himwich found in dogs during absorption of fat a large increase in the cholesterol content of the thoracic lymph. Since the source of this cholesterol most probably was the bile, in which it is entirely in the free form, esterification must have taken place during or before absorption into the lymphatics. Schoenheimer and Hummel found that when cholesteryl oxalate was fed to mice the cholesterol was deposited in the liver and the oxalic acid was excreted by the kidneys. Several alternative explanations refute the idea that this experiment constitutes

38. Cook, 1936. Loizides. Verzár and McDougall. Versé.
39. Verzár and Kuthy. Verzár and McDougall.
40. Hummel. Loeffler.

evidence that cholesterol is absorbed in the free form. For example, the oxalate may have been hydrolized in the intestine and the cholesterol esterified with fatty acids prior to absorption, or the oxalate may have been absorbed as such and hydrolized in the epithelial cells or even in the blood.

Analyses of lymph from the thoracic duct show that cholesterol enters the blood stream by this route rather than by the portal vein. Whereas increases in the cholesterol of the lymph have been demonstrated repeatedly [41] during the absorption of cholesterol, the changes in the portal blood are slight and of doubtful significance.[42] It is doubtful if significant increases in the cholesterol concentration of the portal blood could be demonstrated at the height of the absorption of cholesterol, even if all this went by way of the portal vein, if one takes into account the rate of blood flow, the rate of absorption, possible dilution of the portal blood by intestinal secretions and the limitations on the accuracy of the analysis. The absence of an increase in the cholesterol of the portal blood over that of the arterial blood, therefore, would not constitute evidence against this route being taken during absorption.

The apparent restriction of the absorption of sterols to cholesterol itself suggests that an enzymatic rather than a physicochemical mechanism is responsible. Recently Schramm and Wolff, having found a cholesterol-esterifying enzyme which is activated tremendously by bile salts in the pancreas, offered the following mechanism for the absorption of cholesterol: The fatty acids, set free by pancreatic lipase, are combined with cholesterol under the influence of pancreatic cholesterol esterase and bile; in this form cholesterol is transported into the epithelial cells of the intestine. Here they again are split by the cholesterol-hydrolizing enzyme which Klein found abundantly present in intestinal mucosa. Based on the synergetic action of pancreatic enzymes and bile, this theory explains the necessity for fat and bile in the absorption of cholesterol and is in accord with the known facts, but as yet it has not been put to experimental test.

EXCRETION

Though some sterol is lost by the body in the secretions of the skin (Hueck) and possibly in the urine,[43] the greatest amount is excreted into the intestinal tract. The early views, discussed by Campbell, emphasized the bile as the source of the fecal sterol, but Sperry (1927) found no decrease in the unsaponifiable matter of the feces of dogs

41. Brockett and others. Frölicher and Süllmann.

42. Boyd, 1936 g. Schally. Yuasa.

43. Butenandt and Dannenbaum. Gardner and Gainsborough, 1925.

with biliary fistulas. His results were substantiated by Beumer (1923), Beumer and Hepner (1929), Heinlein (1933) and many others, all of whom observed normal sterol balances with total exclusion of the bile from the intestine. In the feces of a patient with complete closure of the bile duct caused by carcinoma, Bürger and Winterseel (1929 a) found large quantities of sterols which could not be accounted for by the food eaten.

With elimination of the bile as an important source of the fecal sterols, several other possibilities suggest themselves: desquamation of intestinal epithelium, synthesis by intestinal bacteria and direct excretion from the blood. The first theory was examined by Bürger and Oeter. They found the sterol content of the intestinal mucosa extraordinarily low—so low that on a sterol-free diet the excretion of sterols is greater than the amount in the entire mucosa. Similar results were reported by Sperry (1929 c).

The possibility that fecal sterols arise through the synthetic action of intestinal bacteria was suggested by Sperry (1929 b, c), who found appreciable quantities of sterol in fecal bacteria separated by centrifugation. The possibility of adsorption makes the results inconclusive. The absence of sterols in bacteria, shown by various authors,[44] would indicate that they are not products of bacterial metabolism. Bürger and Oeter obtained indirect evidence for the direct transport of cholesterol into the intestinal lumen; in an analysis of segments of intestine, the sterol content of the sigmoid was found almost double that of the duodenum and ileum. The colon, therefore, probably accounts for a large portion of the excreted cholesterol.

There is a difference between omnivora and herbivora in the amounts of sterol excreted. In the herbivora Ellis and Gardner (1912) and Schoenheimer (1929 c), have shown no apparent excretion of sterol, the fecal sterol being the same in amount and composition as the dietary phytosterols. In omnivora and carnivora, on the other hand, large amounts of sterols are in the feces, a greater amount than can be accounted for by the food ingested. In man, Gardner and Fox (1921) estimate the amount of sterol excreted daily to be about 0.6 Gm.—about twice the amount in the food eaten.

Origin of the Saturated Sterols of the Feces.—In omnivora, the fecal sterols, unlike those of the body, are composed mainly of coprosterol, with smaller quantities of the isometric dihydrocholesterol (betacholestanol) and cholesterol. The two isometric saturated sterols, dihydrocholesterol and coprosterol, may be obtained in the laboratory (the former directly, the latter indirectly) by reduction of cholesterol. It is natural to assume that they arise physiologically in the same manner,

44. Anderson and Chargaff. Von Behring. Beumer and Hepner.

though direct conversion in vivo has not been demonstrated. The significance of these saturated sterols has received intensive investigation by Schoenheimer and his co-workers.

Dihydrocholesterol, first isolated from feces by Windaus and Ulbrig, comprises a small, but definite, amount of the sterols of the entire body; from 1 to 5 per cent (Schoenheimer, von Behring and Hummel). Schoenheimer and von Behring proved that dihydrocholesterol was excreted directly by the intestinal mucosa. They allowed the secretions of blind sterile loops of the large intestines of dogs to accumulate for several months. Analysis of the contents for sterols showed only dihydrocholesterol plus a small quantity of cholesterol. These authors also cited an instance of a woman with a blind loop of intestine. The sterile contents contained dihydrocholesterol as the only sterol. Schoenheimer has pointed out that as dihydrocholesterol cannot be absorbed from the intestine, whereas cholesterol is readily absorbed, the elimination of the two sterols in the proportions in which they occur in the blood would be followed by absorption of the latter and accumulation of the former. If this hypothesis is correct, with a proportion of 1 to 5 per cent of dihydrocholesterol in the blood sterols and a fecal content of 100 mg., no less than 2 to 10 Gm. of cholesterol would be excreted and reabsorbed daily.

The presence of coprosterol in the stools cannot be explained on the same basis, however, for this sterol has never been found in the body tissues. Since its discovery by Bondzynski and Humnicki in feces, the view has prevailed that it is produced by the reduction of cholesterol by the bacteria normally present in the large intestine. In spite of much work on this problem, the bacterial origin of coprosterol has not yet been proved conclusively. Attempts by Schoenheimer, von Behring, Hummel and Schindel and by Dam (1934), and also by Bondzynski and Humnicki, to reduce cholesterol in vitro through the agency of putrefactive bacteria were unsuccessful. On the other hand, Dam (1934) and Bischoff observed a slight increase in the saturation of the sterols when feces or colon contents were incubated aerobically or anaerobically. The old observation of Muller that a milk diet, presumably by altering the bacterial flora, inhibits the formation of coprosterol and causes the appearance of cholesterol in the feces often has been quoted in support of bacterial reduction of cholesterol in the intestine. Bürger and Winterseel (1929 b) have not been able to corroborate this hypothesis. They, however, in an investigation of the effects of diet on the composition of fecal sterols, found a fairly constant ratio of cholesterol to saturated sterols in the feces of normal human beings, independent of the amount of cholesterol ingested and of dietary factors which would affect the bacterial flora. Dam's (1934) analyses of fecal sterol show also that the composition is independent of the diet.

The colon unquestionably is the only part of the intestine in which coprosterol is produced. Gardner, Gainsborough and Murray could not isolate coprosterol from intestinal contents discharged from a terminal ileostomy or a colostomy, and Dam (1934) reported the virtual absence of saturated sterols from ileal and jejunal contents.

The chemical mechanism of the reduction of cholesterol to coprosterol has been partially illuminated by Schoenheimer, Rittenberg and Graff through the application of the deuterium indicator method. When added to the basic meat diet of a dog, cholestenone, a direct oxidation product of cholesterol, is converted into coprosterol. If added to a basic diet of dog biscuit, however, it is reduced to cholesterol. When coprostanone, into which deuterium was incorporated, was administered to dogs and man, coprosterol containing deuterium was recovered in the feces. The physiologic significance of these ketones, though never isolated from body tissues and feces, appears to be established.

DEPOSITION AND MOBILIZATION

Though many of the studies of the deposition of cholesterol in body organs have emphasized the pathologic character of this process, evidence indicates that in the liver at least storage of cholesterol is a normal physiologic response to cholesterol overfeeding. The administration of this sterol in small, though greater than normal, amounts of the order of 1 to 2 per cent of the diet causes the deposition of considerable amounts in the liver of rats,[45] cats (Dam, 1934), rabbits[46] and chicks (Sperry and Stoyanoff, 1935 a). Practically all of the increase is confined to the esterified cholesterol, but the free cholesterol increases somewhat if the feeding is protracted (Chanutin and Ludewig, 1933). The storage of cholesterol is greatly enhanced by the simultaneous administration of large quantities of fat, owing probably to the effect of the latter on its absorption. The action of bile salts, previously pointed out, in enhancing the deposition of cholesterol in the liver may be attributed to increased absorption from the intestine.

Opinion differs regarding the quantitative effect of fat on the deposition of cholesterol. Whereas Loizides reported that the deposition of cholesterol ester was proportional to the amount of fat with which the cholesterol was incorporated, Cook (1937) found no greater storage of cholesterol with a 30 per cent fat diet than with one that was 15 per cent fat. Chanutin and Ludewig (1933) observed a greater deposition of cholesterol on a low fat, high carbohydrate diet than on a high fat, low carbohydrate diet.

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45. Channon and Tristan. Chanutin and Ludewig. Cook, 1937. Loizides.
46. Aylward and Stott. Dam, 1934. Weinhouse and Hirsch.

Okey, Gillum and Yokela claimed to have found a difference in susceptibility to cholesterol feeding between males and females. The slightly lower cholesterol content of the liver in female than in male rats was statistically significant.

A new field of investigation in lipid metabolism has been opened in recent years with the discovery that extraordinarily large amounts of fat are deposited in the livers of rats on certain diets, a condition that can be prevented or cured by the administration of various lipotropic substances, of which choline is probably the most outstanding example. As this subject was reviewed recently by Best and Ridout (1939), Channon (1940) and Dragstedt, only the portion of the work related to cholesterol metabolism will be reviewed here.

In contrast with the "fatty" livers produced with high fat, high carbohydrate, low protein or low choline diets or with starvation, in which the changes in the cholesterol content are hardly significant, the "fatty" livers observed after cholesterol feeding have not only a high glyceride content but also considerable quantities, as much as 6 per cent or more of the fresh tissue, of cholesterol esters.

Application of the lipotropic factors, effective in fatty livers of the first type, to the cholesterol fatty liver revealed that the cholesterol esters, as well as the glyceride, were decreased. This effect was obtained with choline,[47] betaine (Dam, 1934), casein (Beeston and co-workers), methionine (Channon, Manifold and Platt) and various analogues of choline (Channon, Platt and Smith). The effect on the cholesterol esters was not so great, however, as on the glyceride fraction. In contrast with the work carried out on rats as a test species, it is of interest that Baumann and Rusch were unable to prevent the deposition of cholesterol in the livers of rabbits by means of choline. It is noteworthy that lecithin exerts the same lipotropic effect as its choline content (Best, Hershey and Huntsman), which might suggest that the lipotropic effect of choline is due to increased synthesis of phospholipid. The apparent constancy of the phospholipid content of "fatty" livers, however, refutes this hypothesis. Yet the studies of Chaikoff and his group, using radioactive phosphorus as an indicator, have shown that the rate of the turnover of phospholipids in the liver is increased by the lipotropic factors, choline, betaine and methionine (Perlmann, Stillman and Chaikoff), and inhibited by cholesterol (Perlmann and Chaikoff). This experimental proof favors the suggestions previously made that the phospholipids play a part in the deposition and the mobilization of fat.

Another type of fatty liver develops in dogs which have been depancreatized and maintained on insulin or in which the pancreatic duct has

47. Beeston and others. Best, Channon and Ridout. Best and Ridout, 1935, 1936. Dam, 1934. Stoesser, McQuarrie and Anderson.

been ligated. The condition of the liver resembles that obtained on diets low in lipotropic factor; i. e., there is a very high glyceride content with little or no change in the cholesterol or the phospholipid content.[48] The condition may be cured or prevented with choline or a lipotropic factor in pancreas (the lipocaic of Dragstedt). According to Chaikoff, the development of "fatty" liver through pancreatic deficiency is accompanied by a decrease in the cholesterol esters of the blood to vanishingly low levels.[49] Such changes in the blood have not been observed in other cases of lipotropic deficiency. The blood lipid levels returned to normal with ingestion of pancreas or choline, though much larger amounts were needed than for removal of the hepatic fat (Entenman, Chaikoff and Montgomery).

Differences in Cholesterol Deposition Between Omnivora and Herbivora.—In omnivora, of which the rat has been the best studied example, the deposition of cholesterol is confined almost completely to the liver.[50] The most thorough study has been carried out by Okey and her co-workers. They found that in spite of the intensely fatty livers of these animals, containing more than twenty times the normal quantity of cholesterol, no effect on the general health was observed. Rats fed 1 per cent of cholesterol withstood poorly balanced and vitamin-deficient diets almost as well as the controls (Gillum and Okey). Pregnancy and lactation were successful (Okey, Godfrey and Gillum), and a group of rats, fed the high cholesterol diet throughout their entire lives, grew as well, lived as long and remained as healthy as control animals (Okey, 1941). Sperry and Stoyanoff (1935 b) studied the effects on rats of long-continued feeding of diets containing 1 per cent cholesterol. The rats receiving a "synthetic" diet containing cholesterol grew less well, ate less food and utilized their food less efficiently than controls receiving the synthetic diets without cholesterol. Such differences were not observed if a diet of natural foods was given in addition to the cholesterol. Undoubtedly, the synthetic diet lacked the lipotropic factors present in the natural foods.

Herbivora, on the other hand, have a poor tolerance for cholesterol. In response to its inclusion in the diet of rabbits in amounts of less than 1 per cent, deposition occurs in all parts of the body, particularly the liver, the adrenal glands and the aorta. Hardly any tissue of the body remains unaffected, deposits of cholesterol occurring in the corneas, kidneys, lungs, spleen, gonads and bone marrow (Schoenheimer, 1924 a).

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48. Aylward and Holt. Kaplan and Chaikoff.

49. Entenman, Chaikoff and Montgomery. Entenman, Montgomery and Chaikoff.

50. Chanutin and Ludewig. Page and Menschick, 1933. Schoenheimer, 1924. Sperry and Stoyanoff, 1935 b.

The reason omnivora may tolerate large quantities of cholesterol without their health being affected in any manner may be that the hepatic cells have the ability to store and probably to destroy large amounts of the substance without damage to the organ (Page, 1941). The demonstration of active excretion of sterols in omnivora (Schoenheimer and von Behring) coupled with the apparent lack of excretion of cholesterol in rabbits and other herbivora [51] may well account for their differences in toleration of exogenous cholesterol.

The metabolism of cholesterol may be summarized briefly as follows: Exogenous cholesterol is absorbed from the intestine with the aid of fats and bile. It passes by way of the lacteal and the lymph ducts into the general circulation, where part is deposited with the depot fat and part enters the liver to be stored, excreted or destroyed. In omnivora the main route of the excretion of cholesterol is the intestine, where it may be reabsorbed or reduced to coprosterol, the form in which it is found in the feces. No excretion of sterols has yet been demonstrated in herbivora except that of the bile, which probably is completely reabsorbed, and that of the small quantities secreted through the skin. The synthesis and the destruction of cholesterol proceed actively in omnivora and herbivora, the relative rates of each process depending on the available dietary cholesterol. The fact that omnivora tolerate large amounts of ingested cholesterol, whereas herbivora show the most widespread effects, is due either to difference in the relative abilities of their livers to store it or to difference in their ability to excrete it.

III. BLOOD CHOLESTEROL IN DISEASE

DISEASES OF THE LIVER

The changes in the cholesterol of the blood in hepatic and biliary disease have received thorough investigation, especially from the clinical standpoint, and though investigators agree essentially in their observations, they differ in their interpretations.

Thannhauser and Schaber, using reliable methods, were the first to observe a diminution in the ratio of esterified to free cholesterol in the blood of patients suffering from disease of the liver. The wealth of early clinical data published since then is confusing because of the variety of analytic methods and because of the inclusion of cases complicated by other diseases. The early work was reviewed by Gardner and Gainsborough (1930 b) and Epstein (1932).

The more recent studies agree that in parenchymatous hepatic disease the serum cholesterol esters reach low levels. Epstein (1937), in a study of 130 cases of jaundice associated with diffuse parenchymal damage due to drugs and other causes, with no biliary obstruction, found

51. Ellis and Gardner, 1912. Schoenheimer, 1929 c.

normal or low values for the total blood cholesterol in 88 per cent. A decrease in the cholesterol esters in 70 per cent paralleled the severity of the clinical symptoms.

Lowered levels of blood cholesterol esters were especially marked in 15 cases of acute, subacute and chronic yellow atrophy. In each, the esters remained low throughout the entire fatal course, at times almost vanishing. The behavior of the blood cholesterol in 35 cases of atrophic cirrhosis is interesting. In 24 cases in which jaundice was absent the ester cholesterol was diminished only slightly, whereas in 11 cases with jaundice the changes in the cholesterol esters resembled those in cases of primary degeneration of the liver.

Similar values for blood cholesterol in cases of hepatic damage were reported by many others.[52] Boyd and Connell (1938) found the lowering of cholesterol esters in nonobstructive jaundice to be only a part of general lipopenia. In 27 cases of parenchymal disease there were, in addition to the lowering of the cholesterol esters, marked decreases in the plasma phospholipids, with slight decreases in the free cholesterol and the neutral fat. Coincident with the lipopenic changes in the plasma, these authors observed increases in the lipid content of the red cells, though not consistently enough for the increases to be statistically significant.

Experimental studies of hepatic injury corroborate the clinical findings. Hawkins and Wright, in dogs with chronic injury of the liver produced by chloroform, observed a drop in the plasma cholesterol esters which paralleled the jaundice and the severity of the clinical condition. When the ester cholesterol dropped to less than 30 per cent of the total, the animals were critically ill, and died when values fell to between 0 and 26 per cent. With subsidence of the jaundice and recovery, the cholesterol esters again became normal or slightly higher. Yet acute injury of the liver from one hour of chloroform-induced anesthesia, accompanied by marked bilirubinemia and the usual toxic symptoms, caused no change in the total cholesterol or in the percentage of esters. Kusui observed a decrease in the blood cholesterol esters after poisoning with toluylendiamine and a return to normal with recovery. In the studies of Chanutin and Ludewig in rats after partial hepatectomy (65 to 75 per cent) the blood cholesterol esters were markedly lower in twenty-four hours but normal after three days. As it was probable that little regeneration had taken place in this time, the authors concluded that the decrease was caused not by removal of the tissue but by dehydration or other damage of the hepatic remnant owing to the shock of the operation.

52. Beumer, 1936. Greene and others. Kusui. Pickhardt and others. White. Deutsch and Maddock.

In contrast to jaundice from damage of the liver, the jaundice associated with biliary obstruction is accompanied by hypercholesteremia (over 300 mg. per hundred cubic centimeters) in 78 per cent of patients in whom the condition was verified at operation or at autopsy. There was no marked correlation between the blood cholesterol and the icteric index, although with relief of the obstruction and diminution of the jaundice the cholesterol was reduced to normal.[53]

Hawkins and Wright observed a marked rise in the total cholesterol but no change in the ratio of free to ester cholesterol in dogs whose bile ducts were ligated. Similar results were reported by Chanutin and Ludewig in rats with ligated bile ducts.

As the ratio of free to total cholesterol normally fluctuates between 25 and 30 per cent, any marked increase in the ratio accompanied by jaundice is strongly suggestive of hepatic damage. This test successfully distinguishes between jaundice of obstructive origin, in which hypercholesteremia with no significant change in the ratio of free to ester cholesterol is found, and the jaundice associated with parenchymal injury, in which the total plasma cholesterol is normal or low and the ratio of free to total cholesterol is increased.[54] Though Bürger (1940) tends to minimize the relation between the partition of cholesterol and hepatic disease, his figures afford good evidence in favor of the relation. It is surprising that this simple test has not been more generally adopted by clinical laboratories. The proportion of free to ester cholesterol is of value in prognosis as well as in differential diagnosis. A continuously decreasing percentage of cholesterol esters indicates severe injury with a bad prognosis; a rising percentage indicates recovery (Greene and co-workers). Because of the multiplicity of hepatic functions, probably no single test will give a reliable picture of this organ, but the cholesterol ester ratio in combination with other clinical and laboratory data is a valuable aid.

Thannhauser and Schaber interpreted the decrease in cholesterol esters in hepatic disease to be a result of inability of the injured cells of the liver to esterify cholesterol, a phenomenon they named *Estersturz*. Many subsequent investigators have agreed, yet there is no conclusive evidence for this hypothesis. Thannhauser claimed to have found a cholesterol ester–synthesizing enzyme in liver. The results of Sperry and Brand, however, were contradictory. They found that an emulsion of rat liver esterified free cholesterol but that the same emulsion incubated with rat serum caused hydrolysis of cholesterol esters. Gardner and Gainsborough (1930 b) expressed the belief that the lower-

53. Thannhauser and Schaber. White, Deutsch and Maddock.

54. Boyd and Connell, 1938. Epstein, 1937. Greene and co-workers. Kusui. White, Deutsch and Maddock.

ing of cholesterol esters may be attributed to the absence of bile from the intestine, which results in nonabsorption of fats and cholesterol. This contradicts several experimental facts: First, as pointed out in the previous section, there is no significant relation between the amount of cholesterol in the diet and the level of cholesterol in the blood; second, nonobstructive jaundice, in which there is no absence of bile from the intestine, causes a decrease in cholesterol ester, whereas complete biliary obstruction actually increases blood cholesterol. Another theory rests on a firmer foundation. Beumer (1935, 1936) found large amounts of cholesterol esters in the livers of patients with acute yellow atrophy and concluded that the decrease in cholesterol esters of the blood in jaundice is a result of their fixation in the diseased liver. No marked variation in cholesterol content between normal livers and those which might have been associated with a decrease in ester cholesterol was noted, however, in a study by Ralli, Rubin and Rinzler or in one by Ralli, Paley and Rubin.

At present the explanation of the marked reduction of blood cholesterol esters in hepatic disease points to the inability of the hepatic cells to synthesize cholesterol esters or to transport them into the blood.

No satisfactory explanation exists for the hypercholesteremia associated with biliary obstruction. Early investigators regarded it as merely a mechanical effect of the obstruction, which impaired excretion of cholesterol. With the finding that the bile cholesterol is completely absorbed, however, and that the main pathway for the excretion of cholesterol is the lower intestine, this explanation does not suffice.

Problem of the Gallstones.—The formation of gallstones was attributed in former days to a disturbance of the metabolism of cholesterol, conforming with the prevailing belief that cholesterol of alimentary or endogenous origin was excreted exclusively with the bile. Hypercholesteremia, it was assumed, resulted in an overburdening of the excretory mechanism, with supersaturation of the bile and consequent precipitation of cholesterol and formation of a concrement.

This theory was questioned when Sperry (1927) demonstrated that the intestine, not the bile, is responsible for the excretion of sterols. The theory had to be abandoned after Campbell, Fox, Gardner and Gainsborough (1930 f) and Dostal and Andrews demonstrated conclusively the lack of any relation between dietary cholesterol or hypercholesteremia and the concentration of cholesterol in the bile.

About ten years ago, Andrews and associates [55] suggested a mechanism for the formation of gallstones which has stood the test of time and been confirmed amply by subsequent research. The formation of

55. Andrews, Dostal, Goff and Hrdina. Andrews, Dostal and Hrdina. Andrews, Hrdina and Dostal. Andrews, Schoenheimer and Hrdina. Dostal and Andrews.

stones, according to this hypothesis, results from a decrease in the ratio of bile salts to cholesterol in the bile. Andrews demonstrated that cholesterol ordinarily is dissolved in the bile through the influence of the bile salts (Andrews, Schoenheimer and Hrdina), probably by formation of loose compounds. This was confirmed subsequently by Spanner and Baumann [56] and by Bashour and Bauman. Of the bile acids present in bile, the best solvent for cholesterol appeared to be desoxycholic acid.

In various species of animals (goats, rabbits, cattle and dogs) the ratio of bile salts to cholesterol was about 100 (Wright and Whipple); in man, it was only 20 to 30. The fact that the critical ratio, below which cholesterol begins to precipitate, is 13 may explain why cholesterol stones are relatively common in man and are apparently absent in animals. A decrease to one-half the normal ratio in the former would lead to precipitation, absent in the latter until an eightfold decrease has occurred.

In studying the factors which might influence the ratio of bile salts to cholesterol, Andrews and associates [57] found that the normal gallbladder does not absorb or excrete bile salts or cholesterol. The absorption of fluid which normally occurs is unaccompanied by any change in the ratio of bile salts to cholesterol or in the ratios of either of these substances to the total solids of the bile. Riegel and co-workers (1931) provided further evidence that neither bile salt nor cholesterol is absorbed or excreted from the healthy gallbladder.

Elman and Taussig and Wilkie and Doubilet claimed to have shown that cholesterol is secreted normally by the mucosa of the gallbladder, but Riegel attributed their results to errors in experimental procedures.

When the gallbladder is injured or inflamed, the wall becomes permeable to bile salts, as shown by Andrews, Schoenheimer and Hrdina; this results in a striking decrease in the ratio of bile salts to cholesterol with a consequent tendency toward precipitation of the latter. This was confirmed by Riegel, Ravdin and Johnston, who observed that dilution rather than concentration of bile occurred as a result of injury to the gallbladder. Bashour and Bauman showed that dilution lessened the solvent effect of the bile salts on cholesterol.

If cholesterol were excreted by the mucosa of the gallbladder, there should be an accumulation of this substance in long-standing cases of obstruction of the cystic duct. In this condition, the bile salts are absorbed, and the cholesterol is usually in the form of a crystalline emulsion; the amount present, however, is no more than can be accounted for by precipitation of the cholesterol in the bile ordinarily present. In fact, the stones found in the obstructed gallbladder usually are com-

56. Pickens, Spanner and Baumann. Spanner and Baumann.

57. Andrews, Dostal and Hrdina. Andrews, Schoenheimer and Hrdina.

posed of calcium carbonate rather than of cholesterol. As Phemister and co-workers have demonstrated, if cholesterol stones are already present, obstruction and bile stasis cause additional precipitations of calcium salts. The foregoing evidence established the role of cholecystitis in the formation of gallstones; yet Andrews, Hrdina and Dostal suggested another factor: that is, the marked lowering of the ratio of bile salts to cholesterol in the hepatic bile in mild disease of the liver.

The correctness of Andrews' views is corroborated by the finding by Andrews, Schoenheimer and Hrdina and by Neumann that the ratio of bile salts to cholesterol in the bile of the gallbladder with concretions is lowered. Doubilet and Colp made similar observations in chronic and acute cholecystitis.

Cholecystitis and hepatic disease, then, appear to help alter the ratio of bile salts to cholesterol, with subsequent precipitation of the latter.

Some investigators have attempted to show that other substances, such as the fatty acids, are important in holding the bile cholesterol in solution. For example, Dolkhart, Lorenz, Jones and Brown, in experiments completely unphysiologic as to p_H and temperature, purported to prove that fatty acids exert a marked solvent effect on cholesterol, being more important than the bile acids in this respect. These investigators, however, have not shown any appreciable solvent effects of fatty acids or their soaps on cholesterol under physiologic conditions. More important, they have not shown that fatty acids are present in normal bile.

Though the factor which determines whether or not cholesterol will be precipitated appears to be the ratio of the substance to the bile acids, the subsequent formation of the concretion depends undoubtedly on a multitude of factors which are not clearly understood at present.

RENAL DISEASE

A survey of the quantitative studies of blood lipids in Bright's disease reveals that high blood cholesterol is characteristic of true nephrosis and the chronic, nephrotic stage of nephritis. Page, Kirk and Van Slyke, in a study of 13 cases of hemorrhagic nephritis, found that the chronic stage of this disease is accompanied by hyperlipemia, involving all the blood lipids. Repeated determinations showed no change in the proportion of each lipid. Similar findings in nephritic patients were obtained by Page and Farr. These investigators contended that nephritic lipemia is uninfluenced by the amount of fat in the diet and, unlike the lipemia associated with myxedema, is not reduced by the feeding of large quantities of thyroxin. They found the ratio of free to total cholesterol unchanged, contrary to the earlier results of Lichtenstein and Epstein, who reported an increase in the proportion of esterified cholesterol in nephrosis and glomerular nephritis.

The origin of nephritic hypercholesteremia and lipemia is obscure. The view sometimes has been expressed that nephrosis is primarily a result of disturbance in lipid metabolism. That similar blood lipid pictures are found in conditions such as nephrosis and glomerular nephritis, which differ distinctly in causation and pathogenesis but are similar as to tubular degeneration, hypoproteinemia and edema, suggests that lipemia is a result rather than a cause of the loss in normal renal function.

That the lipid level is reciprocally related to the protein concentration of the blood is probably of fundamental importance. Inasmuch as the plasma proteins are responsible for the colloidal stability of the lipids, a lowering of protein would presumably cause a decrease in the dispersion of the lipid complex, with resultant agglomeration into larger particles. The decrease in surface available for the action of the lipolytic enzymes hence would lead to a lowered rate of transport and utilization, resulting in accumulation of lipids in the plasma to the point of macroscopically visible lipemia. This crude explanation for nephritic lipemia, though speculative, is supported by the observation that in cardiac edema, which is not necessarily accompanied by hypoproteinemia, there is no increase in the blood cholesterol.[58]

Another explanation for the hypercholesteremia of renal disease has been suggested by Miyazaki. By injecting renal venous serum from a normal rabbit into another normal rabbit, he obtained a rapid fall in blood cholesterol, but a similar injection of arterial or auricular venous blood had no effect. This action was ascribed to a hormone secreted by the renal tubules It not only lowered the blood cholesterol of the normal rabbit but markedly reduced the hypercholesteremia produced by experimental nephritis or nephrectomy. This study, indicating a hormonal disturbance in the lipemia of nephrosis, would be important if verified.

The deposition of granules of anisotropic lipid material (cholesterol esters) in nephrotic kidneys, giving rise to the term "lipoid nephrosis," is probably unimportant, as it resembles the precipitation of lipid substances in other degenerating tissues. Similarly, the cholesteroluria probably does not signify a specific permeability of the injured kidney to cholesterol, which most likely escapes with the protein to which it is normally bound in the plasma (Bruger, 1935 b).

DISEASE OF THE THYROID GLAND

Although the relationship between the function of the thyroid gland and the metabolism of cholesterol and other lipids is unknown, the changes in the blood cholesterol level associated with abnormalities of

58. Bodansky and Bodansky. Port.

this gland are well established. There is general agreement, based on clinical and experimental studies, that high values for blood cholesterol accompany hypothyroidism and that low normal or subnormal values are found in hyperthyroidism.[59]

In myxedema Hurxthal found values up to 500 mg. and Gildea, Man and Peters values as high as 600 mg. per hundred cubic centimeters. These investigators claimed a fair correlation between the disappearance of clinical symptoms, the rise in the basal metabolic rate and the fall in blood cholesterol after thyroid therapy. The relation between the basal metabolic rate and the cholesterol level stressed by many investigators [60] is apparent only when the values in a large number of cases are averaged. When individual instances are examined, many exceptions are found (McGee), a not unexpected result in view of the dependence of the basal metabolic rate on factors other than thyroid activity.

In 21 children with hypothyroidism who were untreated, Wilkins, Fleischmann and Block observed serum cholesterol levels varying from 145 to 160 mg. per hundred cubic centimeters. In contrast with normal children, these children showed great spontaneous variations. The marked instability of the serum cholesterol in myxedema, these authors pointed out, may explain the low values frequently observed in this condition.

In myxedema produced by thyroidectomy and in the spontaneous variety, hypercholesteremia was reported by Gilligan, Volk, Davis and Blumgart; it was observed by Blumgart and Davis after removal of the thyroid gland in the treatment of chronic heart disease. Similar results were reported in cases of thyroidectomy for hyperthyroidism (Man, Gildea and Peters). The rise in the cholesterol concentration was variable, however, and appeared independent of the degree of recovery. The effect of thyroidectomy on the cholesterol level is well illustrated in animal experiments. According to Schmidt and Hughes, in normal dogs thyroidectomy produced marked hypercholesteremia, which reached a peak four to five weeks after operation and which was accompanied by no change in the normal ratio of free to esterified cholesterol and was confined to the plasma.

Most investigators find that in rabbits thyroidectomy is followed by hypercholesteremia but disagree as to the magnitude of this change. Turner, Present and Bidwell observed only a 19 per cent increase, but Westra and Kunde and Fleischmann, Schumaker and Wilkins found increases of about 200 per cent. The effect of thyroidectomy on rabbits with hypercholesteremia of alimentary origin was studied by Turner,

59. Bodansky and Bodansky. Mason, Hunt and Hurxthal. Page, 1937.

60. Cutting, Rytand and Tainter. Epstein and Landé. Gardner and Gainsborough, 1928.

Present and Bidwell. They reported that thyroidectomy caused a 137 per cent rise in the blood cholesterol. In so-called cholesterol-resistant rabbits, moreover, thyroidectomy promptly abolished the resistance and hypercholesteremia promptly ensued.

Hyperthyroidism, according to Hurxthal (1933 b) and others, is accompanied by blood cholesterol levels which on the average are definitely below normal though there is much overlapping of normal ranges. Though studies, reviewed by Man, Gildea and Peters, have indicated normal, even high blood cholesterol levels in hyperthyroidism, nevertheless, the weight of evidence indicates that subnormal values are to be expected to accompany this disease.

In hypothyroidism the variations from normal cholesterol values are large, and small changes in the basal metabolism effect relatively great changes in the cholesterol level. Thus, blood cholesterol determinations in this condition are valuable both in diagnosis and in following the efficacy of treatment. They are especially useful in regard to childhood myxedema, in which the basal metabolic rate is often unstable.[61] In hyperthyroidism, on the other hand, cholesterol levels are almost invariably near or within the normal range and are not changed markedly by decreases in the basal metabolic rate. In this condition, therefore, blood cholesterol determinations probably have little clinical usefulness.

Boyd and Connell (1936) have shown that the hypercholesteremia of myxedema is part of a general hyperlipemia. Of 35 patients with low metabolic rates, 19 showed no improvement with thyroid medication. Their blood lipid levels were not significantly abnormal. Of the other 16, who all improved under thyroid therapy, the levels of blood lipids, fatty acids, free and total cholesterol and phospholipids were well above normal. In 43 patients with hyperthyroidism the same authors observed subnormal levels for all lipid fractions. Despite large individual variations, they concluded that 5 of 6 patients with hyperthyroidism would have lower lipid levels than 5 of 6 normal persons. In hypothyroidism (Gildea, Man and Peters) and hyperthyroidism (Man, Gildea and Peters) Man, Gildea and Peters found that the concentration of phospholipids and of fatty acids paralleled that of the cholesterol in the serum. During therapy the changes in the cholesterol levels were accompanied by proportionate changes in the levels of the other lipids.

The effect of the thyroid gland on the cholesterol and other lipids of the blood is unknown. The levels of these substances undoubtedly are markedly affected by the presence of thyroxin. Administration of whole thyroid or of thyroxin to myxedematous patients lowers the levels of serum cholesterol[62] and other lipids (Gildea, Man and Peters).

61. Gørtz. Wilkins and others.

62. Hurxthal, 1933 b. Lahey.

Thyroxin also lowers the serum cholesterol in normal persons (Gildea, Man and Peters). The effect of the thyroid hormone is well illustrated in animals. In recent experiments Hughes showed that administration of thyroxin or of desiccated thyroid to thyroidectomized dogs reduced the plasma cholesterol if it was already high but had no effect in dogs whose blood cholesterol was low. Massive doses had no greater effect than moderate ones. The observed changes were confined to the plasma.

Because of the lability of the blood cholesterol in herbivora, the effect of thyroxin on the level of this component in rabbits is of interest. Thyroxin, even when administered in large doses, produced only slight decreases in the blood cholesterol of normal rabbits. Thyroidectomized rabbits are much more responsive to it. Administration of thyroxin to intact rabbits in which hypercholesteremia had been induced by cholesterol feeding caused a substantial drop in the serum cholesterol (Turner, Present and Bidwell). A single dose of 1 mg. of the crystalline hormone produced an average 40 per cent decrease. The minimum value was reached in three or four days, the level returning to its previous figure in five to nine days. In thyroidectomized rabbits with alimentary hypercholesteremia, the response differed only in degree, the drop averaging 60 per cent.

Despite the relation between the basal metabolic rate and the blood cholesterol, considerable evidence indicates that the action of thyroid on the blood lipids is not a direct consequence of its effect on metabolism. Hurxthal (1934) has shown that hypometabolism associated with deficiency of the adrenal glands or of the pituitary gland is not accompanied by hypercholesteremia. Large increases in basal metabolism caused by the administration of 2,4-dinitrophenol, furthermore, cause no appreciable drop in the blood cholesterol.[63] These observations suggest that the thyroid hormone does more than regulate the body metabolism. Its effect on the blood and tissue lipids awaits further study.

ATHEROSCLEROSIS

The early identification of cholesterol as a constituent of atheromatous lesions led to speculations regarding its importance in the causation of atherosclerosis. Some investigators have considered the nodular or diffuse accumulations of lipids in this disease as secondary to medial injury (Wells; Duff); others, notably Aschoff, Anitschkow and Leary, have expressed the belief that the deposition of lipids precedes any other changes in the artery.

The influence of the amount and the character of the blood lipids on the development of atherosclerosis has been studied extensively with

63. Cutting and co-workers. Emmer. Grant and Schube. Hurxthal, 1934.

the result only that there has been published much controversial data. Inasmuch as atherosclerosis occurs without definite symptoms until the late stages, the determination of its presence and extent in human patients is difficult. The relationship of the blood lipid levels to this disease can be inferred only by studying conditions, such as hypertension or angina pectoris, closely associated with atherosclerosis.

Mjassnikow (1925) found elevated blood cholesterol levels in angina pectoris, the values varying from 190 to 440 mg., compared with 120 to 170 mg., per hundred cubic centimeters for normal subjects. Davis, Stern and Lesnick reported blood lipid levels frequently elevated in 59 patients with angina pectoris. About 60 per cent had total blood cholesterol levels over 250 mg. per hundred cubic centimeters, whereas only 20 per cent of 54 normal persons had values this high. As a group the patients with angina pectoris had values averaging 260 mg. per hundred cubic centimeters, compared with 218 mg. for the controls. Statistical analysis indicated that the results were significant. Increase of phospholipids and fatty acids also was observed. The ratio of free to total cholesterol remained constant in normal and in diseased patients. In 73 patients with arteriosclerosis obliterans of the legs unassociated with diabetes or hypothyroidism, Barker found that the mean value for blood cholesterol was 263 mg., compared with 218 mg. for normal subjects. Statistical analysis was not carried out, but inspection of the data reveals that the significance of this difference is not great. Poindexter and Bruger observed high average values for the blood cholesterol in 24 cases of arteriosclerosis and in 19 cases of hypertension with arteriosclerosis. The average for these was 250 mg. per hundred cubic centimeters; 33 normal persons had an average value of 195 per cent —statistically a significant difference.

Other investigators have reported normal blood cholesterol levels in clinical conditions associated with arteriosclerosis. Andes, Kampmeier and Adams found no difference in cholesterol level between normal and arteriosclerotic Negroes. In 16 patients with hypertension uncomplicated by nephritis, Page, Kirk and Van Slyke (1936 b) found normal blood lipid levels. In no case was the concentration of any of the lipid fractions (free and total cholesterol, phospholipid and total lipids) outside the range of normal values, nor were the means and standard deviations different from those in normal subjects. In 53 patients with hypertension, Elliot and Nuzum found no significant increase of blood cholesterol. No correlation was noted between the severity of clinically determined arteriosclerosis and the level of the blood cholesterol.

Probably the most important study was made by Landé and Sperry. Recognizing the difficulties in measuring the extent of arteriosclerosis by clinical methods, they determined the postmortem blood cholesterol

of persons who had died suddenly by violence. After comparing the values thus obtained with the degrees of atherosclerosis ascertained by an objective method, namely, the determination of the lipid in the whole aorta, and eliminating all cases complicated by infection or organic disease, they concluded that no relation existed between the blood cholesterol level and the severity of atherosclerosis.

In many, if not most, cases atherosclerosis evidently is unaccompanied by hypercholesteremia. Although the relation of the blood lipids to the development of atherosclerosis remains undisclosed, there is hardly any doubt that the disease can develop in the absence of any abnormality in the levels of blood lipids.

Considerable evidence indicates that atherosclerosis is advanced by hypercholesteremia. The best and most direct proof is had from the results of experimental cholesterol feeding in rabbits. Here the relation of cause and effect is clear and unequivocal. Cholesterol when fed by mouth accumulates in the blood stream; then it is deposited in the aorta in atheromatous plaques closely resembling those of the human disease. Yet other factors are involved besides hypercholesteremia. Although atherosclerosis never was observed in normal rabbits and invariably was found in cholesterol-fed animals, Weinhouse and Hirsch found no relation between the duration and the height of hypercholesteremia and the degree of atherosclerosis. Differences undoubtedly are to be found in the receptivity of the tissue for the blood lipids. These differences also apply to different tissues of the same animal: One rabbit, for example, may have marked atherosclerosis without changes in other organs, and another have little or no atherosclerosis but marked infiltration of the liver or the kidneys.

The effect of factors other than hypercholesteremia in experimental cholesterol atherosclerosis in rabbits was shown by Page and Bernhard. The atherosclerosis following cholesterol feeding can be prevented by simultaneous administration of thyroid extract or organic iodides, their effect, according to Turner, being to prevent the development of hypercholesteremia. Page and Bernhard, however, found that an organic iodine compound having the same protective action against atherosclerosis as thyroid or inorganic iodides raised the blood cholesterol levels of cholesterol-fed rabbits to higher values than those of rabbits fed this substance alone. These iodine compounds evidently protect rabbits against atherosclerosis by affecting the receptivity of the tissue rather than by altering the blood cholesterol.

Clinical evidence that hypercholesteremia is a predisposing factor in atherosclerosis is the extraordinary prevalence of arteriosclerosis in diabetic patients. The close association between high cholesterol and arteriosclerosis in patients with diabetes has been demonstrated by

Gibbs, Buckner and Bloor, White and Rabinowitch. The atherosclerotic tendency among patients with diabetes, according to Duff, is not necessarily due to hypercholesteremia but may be a manifestation of other profound metabolic disturbances in this disease. Xanthomatosis, frequently accompanied by hypercholesteremia, often is associated with cardiac complications of probably arteriosclerotic origin, according to Thannhauser, Montgomery and Osterberg and C. Müller.

Before conclusions may be drawn regarding the influence of hypercholesteremia on the development of atherosclerosis in human beings, it is necessary to know the incidence of atherosclerosis in diseases accompanied by hypercholesteremia, such as hypothyroidism, nephrosis [64] and hepatic diseases. Opinions are contradictory. At present there is no convincing evidence that atherosclerosis results from hypercholesteremia alone. Apparently other factors are necessary. These are reviewed in an excellent summary of the chemical changes in atherosclerosis by Page.

DIABETES

Hyperlipemia is not now an important complication of diabetes. Before the era of insulin, hyperlipemia and hypercholesteremia were characteristic findings. Today, the adequately controlled diabetic patient may be expected to have normal blood lipid levels. An excellent review of the blood lipids in diabetes by Hunt shows that previous to 1924 the blood cholesterol in patients with diabetes averaged well over 300 mg. per hundred cubic centimeters; since 1930 the average has been practically normal: approximately 200 mg. per hundred cubic centimeters.

In cases of diabetic hyperlipemia, extreme values are often found for the blood lipids, concentrations of 20 (Hunt), 23 (Lichtenstein and Epstein) and 26 per cent (Klemperer) having been reported. According to Hunt, the cholesterol in milky, hyperlipemic serum varied from 75 to 1,600 mg. per hundred cubic centimeters. In fat content and appearance such serums resemble cream. The cholesterol, though raised to high levels, does not keep pace with the increase in neutral fat, which accounts for by far the greatest quantity of the lipids in hyperlipemic serum.

It should be emphasized that hypercholesteremia is not directly related to the severity of the diabetes. In a group of 43 cases with an average blood cholesterol value of 557 mg. per hundred cubic centimeters, for example, the average blood sugar was only 240 mg. per hundred cubic centimeters (Hunt). (It more probably is a result of

64. A relationship between the serum cholesterol and atherosclerosis in chronic glomerulonephritis was shown in a recent article by Steiner and Domanski (Am. J. M. Sc. **204**:79, 1942).

lack of control.) As hypercholesteremia (or hyperlipemia) develops gradually in an uncontrolled diabetic patient and subsides slowly with treatment, the cholesterol level of the blood is a good index of the adequacy of treatment.

Hypercholesteremia in a case of diabetic coma (Hunt) is not very serious, and in this case the prognosis is no worse than in other cases of coma. If of short duration, the hypercholesteremia with adequate control subsides without permanent damage. If the hyperlipemia is longer lasting, the prognosis is bad because of the presence or imminence of serious complications. For instance, of 13 uncontrolled diabetic children studied carefully (Hunt), 12 exhibited serious complications, such as arteriosclerosis and xanthomatosis. The seriousness of hypercholesteremia in diabetes, apart from the fact that it signifies disorganization in the metabolism of carbohydrate, lies in the tendency for cholesterol when in high concentration in the serum to be deposited in the tissues, which gives rise to the various lipidoses, atherosclerosis, xanthoma diabeticorum and lipemia retinalis (Thannhauser).

The finding of a low level of blood cholesterol is, however, far more serious. Patients with values of 90 mg. per hundred cubic centimeters or less have a high mortality (Hunt). This condition, fortunately, is rare. It is accompanied in most instances by endocrine disorders and hepatic injuries unrelated to diabetes but to which the diabetes may be a contributing factor.

Lipid Metabolism in Diabetes.—Although acidosis and coma often are associated with high values for blood cholesterol, there is no relation between the height of the blood cholesterol and the degree of acidosis. Thus, in Joslin's clinic (Hunt), in 5 cases with an average carbon dioxide–combining power of 2 volumes per cent the average value for blood cholesterol was 321 mg., and in 14 cases with a carbon dioxide–combining power of 19 volumes per cent it was 311 mg. Man and Peters (1933) also reported the blood cholesterol in 15 cases of diabetic coma to be near or within normal limits. Though most of the studies of lipids in patients with diabetes, especially the clinical, emphasize the hypercholesteremia, the primary derangement is in the neutral fat fraction. This fraction exhibits the greatest fluctuation and is the cause of the milky appearance of the serum. The mechanism by which hyperlipemia is produced in the diabetic patient uncontrolled by insulin is unknown. The proper understanding of this problem undoubtedly must await fundamental chemical and physiologic studies of intermediary fat and carbohydrate metabolism. The complex relations between fats and sugars and their interconversion form one of the most important problems of the biochemist.

Whether or not insulin exerts a direct effect on the blood lipids is not known. In normal men, Bruger and Mosenthal found no con-

sistent effect of the administration of insulin on the blood cholesterol, whereas Randall observed a slight rise in schizophrenic patients treated with insulin. Decreases in blood cholesterol on the administration of insulin to animals were reported by Page, Pasternak and Burt (1931). As these effects were slight and variable, the beneficial effect of insulin on the hyperlipemia of diabetes may be secondary to and dependent on the establishment of normal carbohydrate metabolism.

XANTHOMATOSIS

The term "xanthomatosis" refers to diseases of obscure causation in which abnormal amounts of cholesterol are found in cells of the reticuloendothelial system. Because of its rarity, xanthomatosis may interest the physiologist and pathologist more than the clinician. The cutaneous, cerebral, cardiovascular and visceral symptoms of xanthomatosis presumably are merely local manifestations of similar underlying pathologic changes in the reticular cells. Thus the exophthalmos and the diabetes insipidus of the Schüller-Christian syndrome result from involvement of the dura and the brain; the jaundice associated with cutaneous xanthomatosis in some cases is a result of xanthomatous involvement of the liver or the bile ducts. Clinical, pathologic and chemical features of these diseases are reviewed by Montgomery and Osterberg, Thannhauser and Magendantz, and Thannhauser.

Thannhauser classified xanthomatous disease into primary essential xanthomatosis, due to an intracellular disorder of cholesterol metabolism, and secondary xanthomatosis due to hyperlipemia. Essential xanthomatosis may involve the skin, tendons, blood vessels, endocardium, bile ducts, bones and viscera. Secondary xanthomatous disease occurs only after long-standing hyperlipemia, as in uncontrolled diabetes (xanthoma diabeticorum) or in idiopathic hyperlipemia. Cholesterol may be deposited in the liver, spleen, blood vessels and other organs, as well as in the skin.

The lesions of secondary xanthomatosis differ distinctly from those of the essential type. The characteristic foam cells of the latter are sparse in the former. Extracellular lipid deposits and inflammatory changes are absent in essential and present in secondary xanthomatosis.

The xanthomatous deposits, wherever located, exhibit in common a high lipid content, of which cholesterol is the major component. Eckstein and Wile analyzed a large nodule of xanthoma tuberosum, finding 17.6 per cent lipids, with cholesterol constituting 48.8 per cent of the total. Montgomery and Osterberg, in analyses of tissues in xanthoma tuberosum, reported values for total lipids ranging from 3.7 to 14.0 per cent, with cholesterol ranging from 18 to 64 per cent of the lipid fraction. By far the greatest amount of cholesterol is in the

combined form. Similar values were found in 2 cases of disseminate xanthomatosis. Thannhauser reported values of 5.5 and 1.31 per cent of the wet tissue for cholesterol in the cutaneous lesions of xanthoma tuberosum and xanthomatous biliary cirrhosis, respectively. Data for other lipids in xanthomas are too sparse for generalization.

According to Thannhauser, moderate increases in the blood phospholipid and fatty acids accompany xanthomatosis associated with hypercholesteremia. In 11 instances the serum cholesterol ranged from 210 to 667 mg. per hundred cubic centimeters. The increase was mainly in the ester fraction, except in xanthomatous biliary cirrhosis, in which the esters were low, probably because of hepatic damage.

The origin of the cholesterol in xanthomas is unknown. Though there is general agreement that the disease is a disorder of cholesterol metabolism, it is not clear whether the disturbance is in the blood, in some central organ, in the whole body or in the cells composing the lesion.

Hyperlipemia undoubtedly is often responsible for xanthoma formation. Xanthomatosis may occur with hyperlipemia of long standing, such as that in uncontrolled diabetes (xanthomatosis diabeticorum) or in idiopathic familial hyperlipemia. The condition, designated appropriately by Thannhauser as secondary xanthomatosis, disappears if the blood lipids return to normal by dietary or other treatment. Strictly, it is not a disease but a manifestation of the hyperlipemia. Storage of lipids occurs presumably as a defensive mechanism of the reticuloendothelial system for removing the excess lipids.

Other xanthomatoses, differing histologically from the secondary and designated "primary essential" by Thannhauser, may or may not be associated with high blood lipid levels. Xanthomatosis disseminatum and osseous and visceral xanthomatosis, including the Hand-Schüller-Christian syndrome, are not associated with any abnormalities in the blood lipids. Even in xanthomatoses of the skin (tuberosum and planum) and those involving tendons, blood vessels, the endocardium and bile ducts, usually accompanied by hyperlipemia, remission of the lesions does not depend on decreases in the blood lipids. The xanthomas, usually permanent, may disappear after a long time. Whatever the effect of the blood lipids in the genesis of essential xanthomatosis, it is not their amount alone that is important. In experimental xanthomatosis following cholesterol feeding in rabbits, Weinhouse and Hirsch found no relation between the level of the blood lipids and the amount of xanthomatous involvement of the blood vessels (atheroma) and skin.

The hypothesis suggested by Bloch and by Schaaf and Werner recognizes the importance of the lipid complex as a whole in the

evolution of the lipidoses. The blood lipids, insoluble in the ordinary sense, are held in colloidal suspension by a proper balance between the hydrophobic components, cholesterol and its esters and glycerides, and the hydrophilic phosphatides and possibly other unknown lipids, the whole complex being attached by loose physical or chemical attraction to the proteins of the serum. Any disturbance in the lipid balance or in the proportion of lipid to protein undoubtedly would lead to instability of the colloid, with consequent agglomeration into coarser particles. The avidity of reticulocytes and histiocytes for particulate matter is well known. The coarsely dispersed lipid complex is taken up by these cells, forming then the typical xanthoma cells. The objection to this theory is the absence of abnormality in the blood lipid picture in many cases of essential xanthomatosis.

The foregoing data indicate that although secondary xanthomatosis probably results from an increase in the quantity of cholesterol in the serum or from a disturbance in the colloidal state of the lipid complex, primary essential xanthomatosis may occur without obvious abnormality of the blood lipids.

Thannhauser considered essential xanthomatosis an intracellular disturbance of cholesterol metabolism, the disorder being localized in the xanthoma cells. This view regards the xanthoma cell as a metaplastic reticular cell or histiocyte, the transformation occurring as a result of the metabolic disturbance. The condition is analogous to Niemann-Pick or Gaucher's disease, in which sphingomyelin or cerebrocerebroside, respectively, is stored in the reticuloendothelial cells. Thannhauser designated essential xanthomatosis as metaplastic reticular and histiocytic cholesterosis, Gaucher's disease as metaplastic cerebrosidosis and Niemann-Pick disease as metaplastic sphingomyelinosis.

The nature of the metabolic disorder is unknown. Increase of lipid conceivably may result from increased synthesis within the cell or from impairment of the ability to break down these substances owing to the lack of a hormone or an enzyme. The transport of fatty acids among cholesterol, glycerides, phospholipids and cerebrosides proceeds through exchange reactions, enzymatic in nature. The lack of one of these responsible for a single step in the series may result in the accumulation of one of the lipids. This hypothesis conforms with the often suggested role of the reticuloendothelial system in lipid metabolism. Because of the rarity of xanthomatosis and of reliable lipid analyses of blood and tissues in this disease, etiologic theories are necessarily speculative, and adequate understanding of the disease will have to await further knowledge of the functions of the reticuloendothelial system and its relations to lipid metabolism.

INFECTIONS

Most investigators of the blood cholesterol in acute and chronic infections agree that its concentration increases in all febrile conditions and returns to normal during convalescence.[65] Boyd (1935 j) and Stoesser and McQuarrie found that the decrease was accompanied by comparable decreases in phospholipids. These changes were not dependent on diet or on changes in blood concentration. No constant relation was observed between the rise in the blood cholesterol and the height of the fever, the severity of clinical symptoms or the white cell count. Because of the well known tendency for cholesterol to combine with and detoxify saponins and other toxins, many authors have suggested that the lowering of the blood cholesterol is due to the combination of this lipid with toxins produced by the infectious process (Bills). This hypothesis refutes the facts disclosed by Ewert (1935), who observed in aseptic hyperthermia brought about by chemicals the same lowering as in infectious fevers. It is, furthermore, hard to understand how the extremely minute amounts of toxins circulating in the blood stream during infections could combine with and remove amounts of cholesterol sufficient to create demonstrable hypocholesteremia. The lipopenia of fever more probably is a result of increased metabolism, similar to the blood lipid picture in hyperthyroidism.

TUMORS

Despite the known cancerigenic action of chemical substances closely related to cholesterol, there is no clear physiologic relation between cholesterol metabolism and tumor formation. That certain products of the breakdown of cholesterol in the body cause cancer is an intriguing and challenging hypothesis. To date, however, no evidence has been found of an abnormality in the blood lipids of cancerous persons. Evidence discussed by Roffo and by Knudson, Sturges and Bryan indicates that an increase in the cholesterol content of the skin predisposes to tumor formation in this region. This is apparently a local condition having no connection with the cholesterol content of the blood.

MENTAL DISEASES

The relationship of cholesterol metabolism to mental disease is a well explored subject, but again no clear relation has been established. Cholesterol metabolism may be associated with mental processes, nerve tissues being rich in this substance. All opinions, however, must remain conjectural until fundamental biologic studies establish the role of cholesterol and other lipids in the structure and the function of nerve tissues.

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56. Boyd, 1935 j. Poindexter. Stoesser and McQuarrie.

DISEASE OF THE ENDOCRINE GLANDS

The relationship of the endocrine glands, particularly of the gonads, to the lipid metabolism, is a fascinating subject, and the future undoubtedly will see tremendous advances in this field. At present, an orderly exposition of the relationship of lipids to this rapidly expanding field is difficult.

IV. FUNCTION OF CHOLESTEROL IN THE BODY

The ubiquitous occurrence of cholesterol in the animal kingdom suggests that this substance takes part in fundamental metabolic processes. Despite the number of functions ascribed to cholesterol, no single one has been established with certainty. Bills's outline of the functions which at various times have been attributed to cholesterol will be reviewed briefly.

The chemical similarity between cholesterol and the bile acids, the adrenal hormones, estrogen and androgen and vitamin D suggests several possible functions, but no physiologic relation has yet been established. Cholesterol has been suggested as a "conditioner" of the skin, as an instrument for the conveyance of fatty acids in the body by exchange esterification with glycerides and phospholipids, as an insulator for the myelin sheath and as an important part of the structure of the cell membrane.

The fact that cholesterol has a neutralizing action against hemolytic substances, such as snake venoms, saponins and bacterial toxins, suggests an important function, but again there is no proof that cholesterol in the blood or the tissues serves such a purpose.

Regardless of the function of cholesterol in the body, it must be emphasized that its action probably is associated closely with that of the other lipids. As the previous pages have shown, any change in the blood cholesterol is accompanied invariably by a comparable one in the phospholipid and glyceride components. Before the changes in the blood cholesterol in the presence of disease may be understood, considerable study will have to be devoted to the functions and the metabolism of cholesterol and the relations of this to the other lipids.

Complete blood lipid analyses in cases of metabolic disease would be highly desirable; such determinations are long and tedious, however, and too complex for the average technician to master. Hence this type of investigation can be carried out at present only by expert chemists. The adoption of complete lipid analyses as a routine hospital laboratory procedure must await the discovery of better and simpler methods.

Reliable and simple methods for the determination of free and esterified cholesterol are available. Coupled with a knowledge of the

normal level and the changes to be expected under physiologic conditions, blood cholesterol values may contribute greatly in the diagnosis of disease.

One reason for contradictory reports is the frequent employment of so-called clinical methods of analysis, which are grossly inadequate with respect to completeness of extraction or analytic precision. A detailed criticism of each study is, however, far beyond the scope of this review. Doubtless better agreement will come with more general adoption of reliable analytic procedures. Another source of conflict is more apparent than real; that is, conclusions often have been based on observed differences within the order of magnitude of the experimental error of the method employed.

There is a strong probability, however, that the lack of agreement, so characteristic of data in the field of the blood lipids, may be caused by the lack of control of one or more hitherto unrecognized factors. For example, the effect of variations in the water content of the blood which occur under certain conditions hardly has been considered. Determinations of cholesterol paralleled by measurement of the specific gravity of the fluid possibly may yield a truer picture of the blood cholesterol than exists at present.

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Notes and News

Appointments, Etc.—Alan N. Drury, Huddersfield lecturer in special pathology at the University of Cambridge and a member of the scientific staff of the Medical Research Council, will become director of the Lister Institute, London, March 31, on the retirement of Sir John C. G. Ledingham.

William F. Petersen has resigned as professor of pathology in the University of Illinois College of Medicine.

Awards.—The Chicago Section of the American Chemical Society has awarded its thirty-second Willard Gibbs Medal to Conrad A. Elvehjem, professor of biochemistry in the University of Wisconsin, in recognition of his original contributions in biochemistry.

Leslie T. Webster, of the Rockefeller Institute for Medical Research, received the fifth annual award of the Dog Writers Association for his work on the diseases of dogs, especially rabies.

Society News.—The American Association of Pathologists and Bacteriologists and the American Association of Immunologists have decided to omit their 1943 meetings.

Deaths.—Leo Zon, passed assistant surgeon of the United States Public Health Service and pathologist to the United States Marine Hospital in Baltimore, has died.

Edgar Allen, professor of anatomy in Yale University, outstanding investigator in anatomy, physiology and endocrinology, died on February 3 at the age of 50 years.

Children's Tumor Registry.—Under the auspices of the American Academy of Pediatrics, a registry of tumors in childhood has been established in the Memorial Hospital for the Treatment of Cancer and Allied Diseases, New York. The material will include all forms of cancer as well as "certain benign tumors" in children less than 15 years of age.

Public Health Research Institute of the City of New York.—This is said to be the first institute of its kind. It is a nonprofit institution for fundamental research in medicine, public health and other problems, authorized by the state legislature. O. A. Bessey, department of pathology at Harvard Medical School, becomes director in the place of Ralph Muchenfuss, who has entered war service.

Bequest for Cancer Research.—It is reported that about $2,000,000 has been bequeathed to the Lankenau Hospital, Philadelphia, for cancer research.

Books Received

Report of the Committee on Tuberculosis in War-Time, Special Report Series No. 246. Privy Council Medical Research Council. Pp. 36. Price 9d. London: His Majesty's Stationery Office, 1942.

The Hemorrhagic Diseases and the Physiology of Hemostasis. Armand J. Quick, Ph.D., M.D., associate professor of pharmacology, Marquette University School of Medicine, Milwaukee, Wis. Vol. 8. Pages 340, with 23 figures. Price $5. Springfield, Ill.: Charles C Thomas, Publisher, 1942.

Human Embryology. Joseph Krafka Jr., M.D., Ph.D., professor of microscopic anatomy, University of Georgia School of Medicine. Pp. 395, with 222 illustrations. Price $4.75. New York and London: Paul B. Hoeber, Inc., 1942.

A concise textbook of human embryology designed to give the medical student the essentials of the subject in the time now allotted to it by the curriculum.

Autonomic Regulations: Their Significance for Physiology, Psychology and Neuropsychiatry. Ernst Gellhorn, M.D., Ph.D., professor of physiology, University of Illinois College of Medicine. Pp. 373, with 80 illustrations. Price $5.50. New York: Interscience Publishers, Inc., 1943.

Ovarian Tumors. Samuel H. Geist, M.D., attending gynecologist, Mount Sinai Hospital; clinical professor of gynecology, College of Physicians and Surgeons, Columbia University. Pp. 527, with 266 figures. Price $10.50. New York: Paul B. Hoeber, Inc., 1942.

Fundamentals of Immunology. William C. Boyd, Ph.D., associate professor of biochemistry, Boston University School of Medicine; associate member, Evans Memorial, Massachusetts Memorial Hospitals, Boston. Pp. 446. Price $5.50. New York: Interscience Publishers, Inc., 1943.

This is an excellent book for scientists and graduate students who are prepared to delve deeply into the science of immunology, but it is rather advanced and elaborate to serve as an introductory textbook for the undergraduate medical student. This statement applies particularly to the chapters on the basic principles of immunology.

Familial Nonreaginic Food-Allergy. Arthur F. Coca, M.D., medical director, Lederle Laboratories. Pp. 160, with 20 tables and 12 charts. Price $3. Springfield, Ill.: Charles C Thomas, Publisher, 1943.

The author describes tachycardia as associated with morbid reactions to various kinds of food. Tachycardia may serve to identify the particular food it concerns in a given case.